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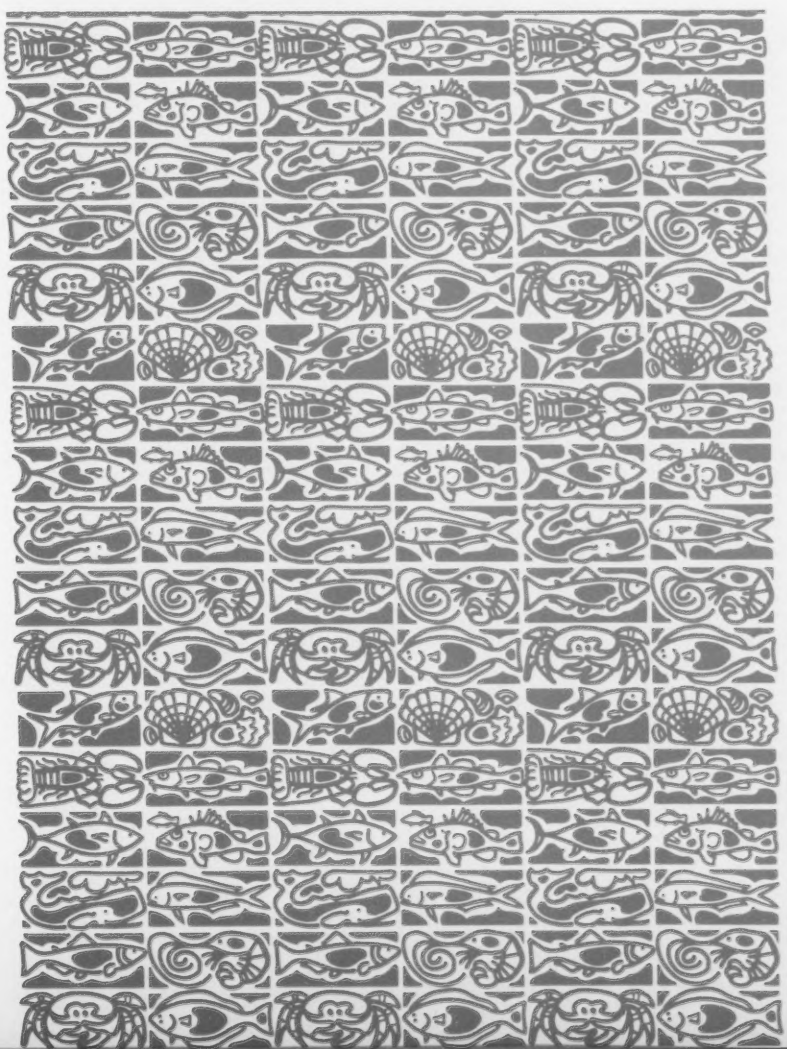
# Fishery Bulletin



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# Fishery Bulletin

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# Fishery Bulletin

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# Stock discrimination of school mackerel, *Scomberomorus queenslandicus*, and spotted mackerel, *Scomberomorus munroi*, in coastal waters of eastern Australia by analysis of minor and trace elements in whole otoliths

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**Abstract.**—The concentrations of 11 elements in individual whole sagittal otoliths from school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*Scomberomorus munroi*) collected in east coast waters of Queensland, Australia (16°S to 28°S) were determined by using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Spatial and ontogenetic variation in otolith elemental composition was examined to make inferences about the stock structure of these species. Stepwise canonical discriminant analyses of the concentrations of barium, potassium, magnesium, manganese, sodium, phosphorus, sulphur, and strontium were used to differentiate groups of fish. These analyses identified an optimum grouping of at least two stocks of school mackerel and a single stock of spotted mackerel in the study region, although our results showed that the most informative comparisons were made among fish of the same year class. The age of fish in collected samples produced strongly significant effects on mean elemental composition of otoliths. These patterns offered independent support for hypotheses about stock structure from previous tagging, catch monitoring, ageing, and reproductive studies. Discrimination between school and spotted mackerel stocks will enable the species to be managed on the basis of stock structure throughout their east coast distribution.

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Spanish mackerels of the genus *Scomberomorus* have restricted ranges and are found in coastal waters of eastern Australia within the confines of the 20°C isotherm (Munro, 1943). Throughout their range they form the basis of important commercial, recreational, and artisanal fisheries, which are characterized by mixed gear types, seasonal availability at widespread points of capture, and movement or migration through various coastal jurisdictions (Trent et al., 1987; Johnson et al., 1994). These traits make it necessary, but difficult, to identify the stock structure of *Scomberomorus* species for effective fisheries management.

Tag-recapture information, catch and effort data, reproductive studies, larval surveys, and protein electrophoresis have recently been used to determine the stock structure of king mackerel (*Scomberomorus cavalla*) in the Gulf of Mexico and Atlantic Ocean (Sutter et al., 1991; Johnson et al., 1994). In northern Australia, stock structure of the large narrow-barred Spanish mackerel (*Scomberomorus commerson*) has been inferred from protein electrophoresis (Shaklee et al., 1990), whereas hypothetical stock structures have been developed for the "lesser" mackerels—school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*Scom-*

*beromorus munroi*)—from tagging, catch data, and ageing and reproductive patterns (Begg et al., 1997; Begg and Sellin, 1998; Begg, in press).

School and spotted mackerel co-occur in coastal waters of northern Australia and southern Papua New Guinea (Collette and Russo, 1984). Within Australian waters they are a major part of set gillnet and ring-net commercial fisheries and support popular recreational fisheries, especially in the region from Moreton Bay to Townsville (Fig. 1). Between 1992 and 1995 the Queensland commercial catch averaged 160 metric tons, and the recreational landings were estimated to be at least half as large as their commercial counterparts (Cameron and Begg[1]). Tag-recapture data indicate that school mackerel move small distances, in contrast to spotted mackerel that migrate annually along the Australian east coast (Begg et al., 1997). Both species reach 100 cm in fork length (LCF, tip of snout to fork of tail) and 8 kg in weight (Collette and Russo, 1984), and first maturity occurs within 2 years of age, between 35 and 50 cm LCF (Begg, in press). Along the Queensland east coast, school mackerel spawn between October and January, whereas spotted mackerel spawn from August to October in northern Queensland waters (Begg, in press). A number of separate school mackerel stocks are thought to exist along the Queensland east coast, whereas spotted mackerel may form a single stock (Begg et al., 1997; Begg and Sellin, 1998; Begg, in press).

The lack of a direct and widely applicable technique to trace larval dispersal has encouraged development of a wide range of methods to deduce stock structure (Thresher et al., 1994) including analysis of the elemental composition of otoliths (Mulligan et al., 1987; Campana et al., 1994; Thresher et al., 1994; Edmonds et al., 1995; Proctor et al., 1995; Kalish et al., 1996). Inference about stock structure from these studies presumes that geographically distinct stocks possess a characteristic elemental composition that reflects the chemical constituents of the environment in which the fish reside.

The elemental composition of otoliths has been measured by analysis of dissolved whole otoliths and by scanning otolith cores or sections with a variety of different electron beam and laser probes (Gunn et al., 1992; Campana et al., 1994). Solution-based or "bulk" chemical analyses of otoliths have the benefits of greater replication in sample sizes through reduced time and cost for preparation, calibration, and measurement (Campana and Gagné, 1995; Campana et al., 1995). However, bulk analysis of whole otoliths incorporate an integrated chemical signal that represents the entire ontogenetic history and home range of individual fish in a nonlinear form that is governed by the growth of the otolith matrix in terms of both rate of deposition and volumetric considerations. Consequently, the mean composition of an otolith in a bulk analysis could be disproportionately dominated by the deposition of material prior to capture, preventing fine-scale resolution of individual affinities with spawning grounds and larval habitats (Campana et al., 1995).

The present study was conducted to determine the utility of elemental analysis of whole otoliths in studying the prevailing hypotheses of a multistock complex for school mackerel and a single stock for spotted mackerel in coastal waters of eastern Australia. Analyses were specifically structured to account for and distinguish the influence of fish age on elemental composition of otoliths. Interpretation of stock structure in this study relates to Ihssen et al.'s (1981) definition of a stock as "a group of randomly mating, reproductively isolated individuals of a single species with temporal or spatial integrity."

[1] Cameron, D. S., and G. A. Begg. 1998. Fisheries biology and interaction in the northern Australian small mackerel fishery. Fisheries Research and Development Corporation 92/144. Unpublished manuscript.

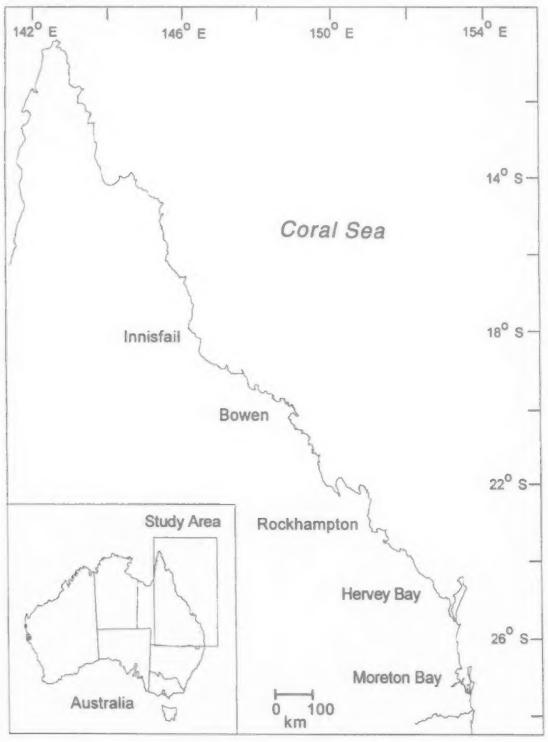


**Figure 1**
Sampling locations for school and spotted mackerel.

## Materials and methods

### Otolith chemistry

School and spotted mackerel were collected monthly from commercial and recreational fisheries along the Queensland east coast, south of 16°S, between June 1992 and February 1995 (Fig. 1). School mackerel were sampled from three geographic areas and spotted mackerel from four (Table 1). Samples were obtained from independent collections at each area, usually representing fish caught from several different schools. Specimens were kept on ice, then frozen until length to caudal fork (LCF, mm) was measured, sex was determined, and both sagittae were removed, washed, dried, and weighed. Fish were aged from whole and sectioned sagittae according to Begg and Sellin (1998).

Precautions were taken to prevent confounding of results based on sexual maturity, size, and age. All samples were sexually mature, between the lengths of 492 mm and 790 mm LCF (Table 1). Fish of the same age (2-year-old school mackerel; 3-year-old spotted mackerel) were selected to minimize age-related variation amongst samples. Additional samples of 1-year-old school mackerel from Rockhampton and 1-year-old spotted mackerel from Moreton Bay and Hervey Bay were examined in relation to the older samples from the same areas to investigate the effects of age on the concentrations of elements in sagittae.

Whole otoliths used in the analysis were cleaned of oil and organic residuals in an ultrasonic bath, rinsed in distilled water, and oven-dried at 60°C for 15 hours. Otoliths were dissolved in 0.5 mL of a Lefort aqua regia solution (75% nitric and 25% hydrochloric acid) and placed in an AIM500 block digester at 90°C for 30 minutes. After cooling, the solutions were made up to 10 mL with distilled water and analyzed with a Varian Liberty 220 inductively coupled plasma atomic emission spectrometer (ICP-AES). Eleven elements (Ba, Ca, Fe, K, Li, Mg, Mn, Na, P, S, and Sr) were analyzed from each otolith, and the concentrations were standardized for individual otolith weights.

Minimum detection limits for the elements were determined by averaging a series of acid blank solutions for both species that were regularly interspersed throughout the samples. The minimum detectable solution concentration for each element (mg/L) was converted to the minimum detectable otolith concentration (mg/Kg) by using the solution volume and the smallest whole otolith weight actually analyzed. This procedure ensured that the most conservative (largest) minimum detection limits were used in the analyses. Three standard solutions were included regularly among the samples to calibrate measurements. The standards covered the entire weight range of otolith material used in the analyses. Only those elements measured in concentrations above the minimum detection limits of the ICP-AES were used in the statistical analyses.

### Data analysis

The chemical composition of school and spotted mackerel whole otoliths sampled from the different areas was analyzed to identify the optimum groupings of fish in order to make inferences about stock structure. All data were examined for normality and homogeneity of variances, and concentrations of Mg and Na in school mackerel otoliths, and K, Mg, Na, and P in otoliths of spotted mackerel, were $\log_e$-transformed to enable statistical analysis. Analysis of covariance (ANCOVA) was used to determine the effect of area of collection (the main effect) on the concentration of each element, while controlling for effects due to fish length (the covariate). Elemental concentrations for which area-by-length interactions were significant were not included in further analy-

**Table 1**

Data from school and spotted mackerel samples used in otolith trace element analyses.

| School mackerel | | | | | Spotted mackerel | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Date | Area | *n* | Length range (mm) | Age (yr) | Date | Area | *n* | Length range (mm) | Age (yr) |
| Nov 1993 | Moreton Bay | 19 | 512–614 | 2 | Feb 1994 | Moreton Bay | 30 | 555–685 | 1 |
| Jun 1993 | Rockhampton | 25 | 492–574 | 1 | Feb 1994 | Hervey Bay | 28 | 544–715 | 1 |
| Jun 1993 | Rockhampton | 19 | 552–640 | 2 | Dec 1993 | Hervey Bay | 29 | 552–790 | 3 |
| Aug 1993 | Bowen | 22 | 545–632 | 2 | Aug 1993 | Bowen | 32 | 605–670 | 3 |
| | | | | | Jul 1993 | Innisfail | 28 | 585–665 | 3 |

ses. Elements for which concentrations were significantly correlated with length were corrected for statistical analysis as

$$AC = C - rL,$$

where $AC$ = the corrected concentration, adjusted for fish length;
$C$ = the concentration of a given element (mg/Kg) for a fish of fork length $L$ (mm); and
$r$ = the regression coefficient or the "common slope" for the covariate length (Edmonds et al., 1989).

ANCOVA and other subsequent analyses were also performed with otolith weight as the covariate, but they showed lower power in discriminating among groupings of fish. This lower discriminatory power was probably a result of the greater measurement errors in obtaining otolith weights in relation to measurement of fish length. Therefore, concentrations of elements corrected for length only were used in the final discriminant analyses.

Multivariate analysis of variance (MANOVA) was used to compare the mean corrected elemental concentrations of samples from the different areas. One-way fixed effects and unbalanced analyses of variance (ANOVA) were then used to compare concentrations of individual elements in samples from different areas to explain differences detected by the MANOVAs. Significance levels were corrected for multiple testing with the Bonferroni adjustment factor. Tukey's studentized range (HSD) tests were used for *a posteriori* comparisons of the different areas for each significant element.

Principal component analysis (PCA) of the corrected concentrations of elements was used to investigate grouping patterns by area and fish age. ANOVA was performed on the first (PC I) and second (PC II) principal components to test differences among proposed groups. Forward stepwise canonical discriminant analyses were used to detect differences in the length-corrected chemical composition of otoliths for school and spotted mackerel from the different areas. The significant ($P<0.05$) canonical variates (CV) provided by each analysis represented the optimal combination of areas and elements that provided the best overall discrimination between the samples. The pooled-within-groups correlations of significant length-corrected elemental concentrations with each canonical variate approximated the contribution of the respective element to the discrimination between areas. Wilks's lambda denoted the statistical significance of the discriminatory power of the overall model, ranging from 1.0 to 0.0 (perfect discrimination). ANOVA and HSD were used on the significant discriminant functions to test pairwise comparisons among groups detected by the discriminant analysis. Jack-knifed cross-validation procedures were used to give an unbiased estimate of classification success (SYSTAT, 1997).

## Results

### School mackerel

We measured the concentration of 11 trace and minor elements in whole otoliths of school mackerel (Fig. 2). Iron and lithium were highly variable, often at concentrations below the detection limits of the spectrometer, and were excluded from data analyses. Calcium was also excluded because its high concentration (31.4–44.7%) would mask contribution of trace elements in the data analyses. The remaining elements (Ba, K, Mg, Mn, Na, P, S, and Sr) were present in measurable quantities, suitable for statistical analyses. ANCOVA indicated that none of these eight elements were correlated with fish length for school mackerel. However, there was a significant interaction in the concentration of Ba with area of collection and fish length (ANCOVA, $F=2.75$, df=3, 76, $P<0.05$); therefore the Ba data were not used in the discriminatory analyses. This interaction was caused by an inverse relationship between Ba concentration and fish length for school mackerel from Bowen, whereas samples from the other areas all showed positive correlations between Ba concentration and fish length.

School mackerel from each age class in each area were found to have significantly different mean elemental composition of otoliths (MANOVA, Pillai's trace=1.013, $F=5.54$, df=21, 228, $P<0.0001$). Mean concentrations of individual elements, except for S and Sr, varied significantly among school mackerel from the different areas and between different age classes (Fig. 2). Sodium, P, and Mg concentrations differed the most among the study areas, and there were significant differences in concentrations of Na and P between fish from Bowen and those from Rockhampton and Moreton Bay. There were also significantly higher concentrations of K in otoliths of Bowen fish, compared with those from Rockhampton (HSD, $P<0.05$). No significant differences in elemental composition of otoliths were detected between 2-year-old school mackerel from Rockhampton and Moreton Bay. In contrast, significant differences were found for Mg, Mn, Na, and P concentrations between 1- and 2-year-old fish from Rockhampton, suggest-

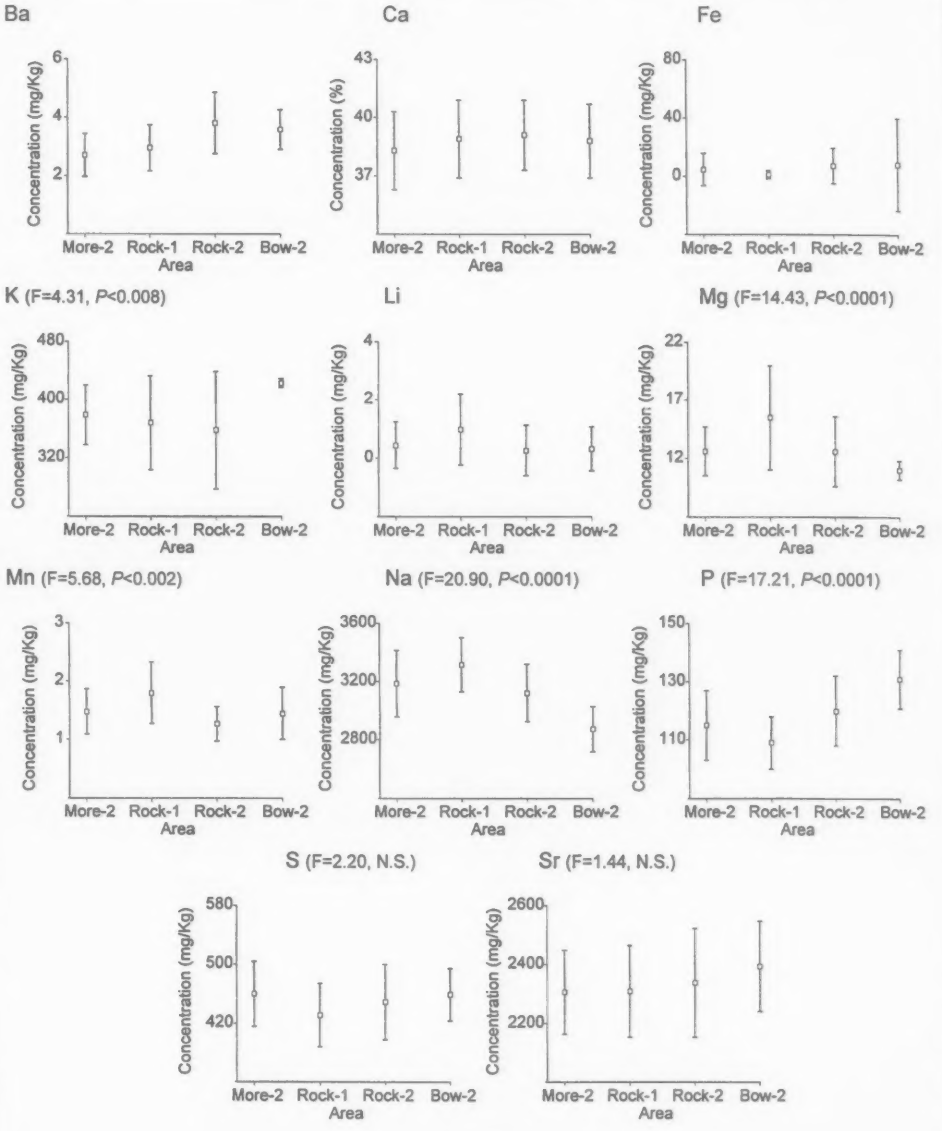


**Figure 2**

Mean elemental concentrations (± standard deviation) of school mackerel otoliths sampled from the different areas and *F*-values determined from ANOVA showing significant elements among samples (df=3, 80; N.S.=nonsignificant result). Minimum elemental detection limits of the inductively coupled plasma atomic emission spectroscopy (ICP-AES) (all units are in mg/Kg, except Ca which is measured as a %): Ba = 0.78; Ca = 544; Fe = 23.9; K = 353; Li = 15.0; Mg =10.9; Mn = 1.10; Na = 1250; P = 54.1; S =191; Sr = 3.24.

ing a strong effect of age at collection in measurement of the elemental composition of otoliths in this area (HSD, $P<0.05$). Sodium showed the most significant differences in these univariate analyses (Fig. 2) and was selected as the common variable for scatterplots in Figure 3. Scatterplots of the relationships between Na and the other significant elemental concentrations revealed similar spatial patterns

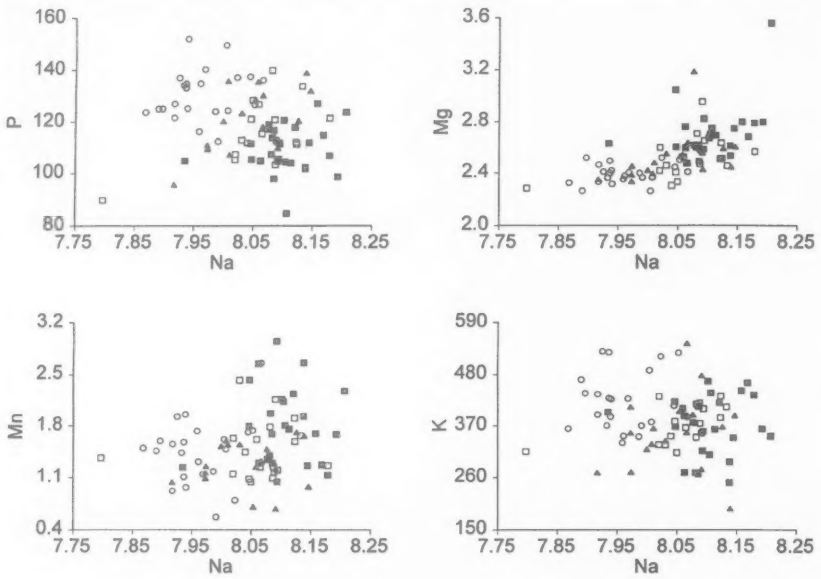


**Figure 3**

Scatterplots of significant elemental ratios showing grouping patterns of area and age school mackerel samples. Open circle = Bowen 2-year-old fish; open square = Moreton Bay 2-year-old fish; solid square = Rockhampton 1-year-old fish; and solid triangle = Rockhampton 2-year-old fish. Mg and Na are $\log_e$-transformed concentrations.

in the otolith chemical composition among samples from the different areas (Fig. 3). The ratio of Na to P concentrations provided the best discrimination among areas, with Bowen samples distinguishable from Rockhampton and Moreton Bay, and samples from Rockhampton and Moreton Bay once again overlapping in their elemental composition (Fig. 3).

Principal component analysis provided further support for the strength of these proposed grouping patterns (Fig. 4). Bowen samples were distinguished from those from the other areas mainly on the first principal component (PC I) where most of the variation was explained by differences in P concentration, whereas Na and Mg contributed most to the variation in the second principal component (PC II). These two components described almost 57% of the total variation in the data. Rockhampton and Moreton Bay samples showed a similar distribution of PC scores, in contrast to samples from Rockhampton that showed some degree of separation between 1- and 2-year-old fish. ANOVA supported these patterns with significant differences detected for both the PC I ($F$=24.89, df=3, 80, $P$<0.0001) and PC II scores ($F$=6.30, df=3, 80, $P$<0.0007) among the samples. Bowen samples had significantly higher PC I scores

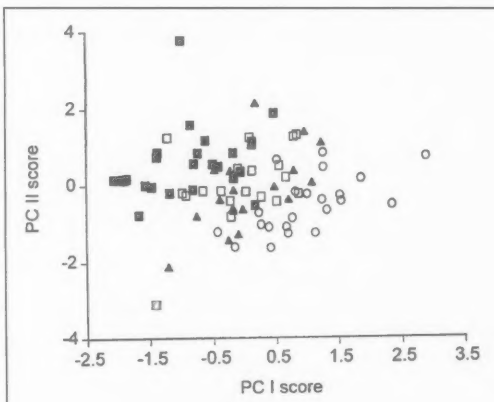


**Figure 4**

Principal component (PC) analysis of school mackerel otolith elemental concentrations indicating grouping patterns. Open circle = Bowen 2-year-old fish; open square = Moreton Bay 2-year-old fish; solid square = Rockhampton 1-year-old fish; and solid triangle = Rockhampton 2-year-old fish.

than those from Rockhampton and Moreton Bay, and no differences were found between 2-year-old fish

from Rockhampton and Moreton Bay. The samples of 1-year-old fish from Rockhampton had PC I scores that were significantly different from all the 2-year-old samples, and PC II scores that also were significantly different from the other samples, but not from Moreton Bay (HSD, $P<0.05$).

Analyses indicated that school mackerel samples are best separated into three groups: 1) Bowen 2-year-old fish; 2) Moreton Bay and Rockhampton 2-year-old fish; and 3) Rockhampton 1-year-old fish (Wilks's lambda=0.195) (Fig. 5). Similar discriminant patterns were observed when the "significant" elements (K, Mg, Mn, Na, P; Wilks's lambda=0.214) were used in isolation. Significant differences in the discriminant scores were found between these groups for both the first canonical variate (CV I) (ANOVA, $F=74.10$, df=3, 80, $P<0.0001$) and the second (CV II) (ANOVA, $F=6.61$, df=3, 80, $P<0.0001$). Like the results of the PCA analyses, Bowen samples had significantly different CV I scores than those from Rockhampton and Moreton Bay; no differences were found between Rockhampton and Moreton Bay 2-year-old fish; and Rockhampton 1-year-old samples had CV I scores significantly different from all the other groups. The Rockhampton 1-year-old samples also had CV II scores that were significantly different from the other groups, with the exception of fish from Bowen (HSD, $P<0.05$).

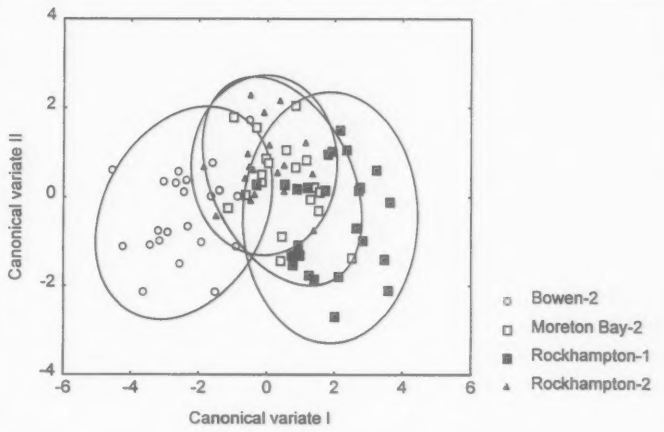


**Figure 5**

Discrimination between school mackerel samples based on the concentrations of 7 trace elements (± 95% confidence ellipses around the sample mean for each area).

Variation in Na, P, and Mg concentrations were primarily responsible for the separations apparent among the samples (89%) according to the first canonical variate (Table 2). Bowen fish tended to have lower Na and Mg and higher P concentrations than Moreton Bay and Rockhampton samples (Fig. 2). Approximately 62% of the school mackerel samples were classified into their correct groupings, indicating an overlap in otolith composition of Rockhampton and Moreton Bay samples (Table 3). Overall classification success increased to 77% when these areas were pooled, and individual classification rates improved for Bowen 2-yr-old fish (86%), Moreton Bay and Rockhampton 2-year-old fish (70%), and Rockhampton 1-year-old fish (80%), providing further support for the notion of three distinct groups in elemental composition of otoliths.

**Table 2**

Discrimination between samples of school mackerel determined by the pooled within-group correlations of elemental concentrations with the significant ($P<0.05$) canonical variates I and II, and the cumulative proportion of the explained variance accounted for by each function for seven elements (Wilks's lambda=0.195).

| | Canonical variate | |
|---|---|---|
| Element | I | II |
| Na | 0.53 | 0.07 |
| P | –0.48 | 0.02 |
| Mn | 0.17 | –0.72 |
| K | –0.20 | –0.40 |
| Mg | 0.42 | –0.38 |
| S | –0.13 | 0.24 |
| Sr | –0.13 | –0.11 |
| Cumulative proportion | 0.89 | 0.97 |

## Spotted mackerel

The same suite of 11 elements that were used for school mackerel were measured and analyzed for spotted mackerel (Fig. 6). Iron, Li, and Ca were excluded from statistical analyses for the same reasons as they were for school mackerel. Concentrations of Na (ANCOVA, $F=14.02$, df=1, 141, $P<0.0003$; $r=-0.0004597$) and Sr (ANCOVA, $F=6.03$, df=1, 141, $P<0.02$; $r=-0.9227$) in spotted mackerel otoliths were highly correlated with fish length; therefore the data were corrected for length with the respective regression coefficient for the length covariate. Manganese was not used in statistical analyses because a significant interaction in concentrations existed be-

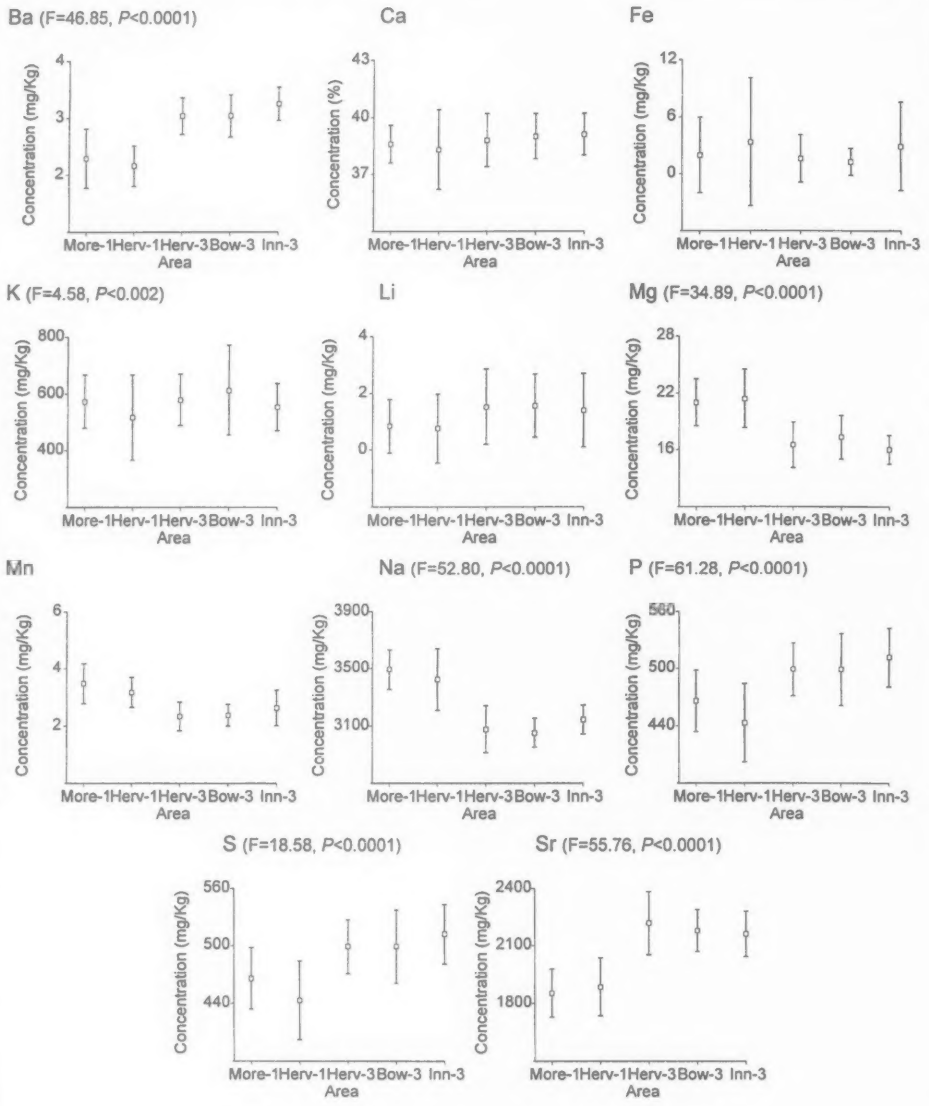


**Figure 6**

Mean elemental concentrations (± standard deviation) of spotted mackerel otoliths sampled from the different areas and *F*-values determined from ANOVA showing significant elements among samples (df=4, 140). Minimum elemental detection limits of the ICP-AES (all units are in mg/Kg, except Ca which is measured as a %): Ba = 0.78; Ca = 544; Fe = 23.9; K = 353; Li = 15.0; Mg = 10.9; Mn = 1.10; Na = 1250; P = 54.1; S = 191; Sr = 3.24.

tween area of collection and fish length (ANCOVA, $F$=3.87, df=4, 137, $P$<0.005). Manganese concentration increased linearly with fish length for Hervey Bay 1-year-old fish, in contrast with the other samples that all showed negative correlations.

Spotted mackerel samples from each area and for each age were found to have significant differences in mean elemental composition of the otoliths (MANOVA, Pillai's trace=1.316, $F$=9.59, df=28, 548, $P$<0.0001). Little variation was evident in mean ele-

**Table 3**

Jack-knifed cross-validation classification matrix of the frequency of assigned cases in each area (and age) used to differentiate school mackerel samples.

| | | Classification of individual school mackerel by area | | | |
|---|---|---|---|---|---|
| Area | Correct (%) | Bowen (2) | Rock (2) | Rock (1) | Moreton (2) |
| Bowen (2) | 82 | 18 | 3 | 0 | 1 |
| Rock (2) | 50 | 2 | 9 | 1 | 6 |
| Rock (1) | 64 | 0 | 3 | 16 | 6 |
| Moreton (2) | 47 | 1 | 4 | 5 | 9 |
| Total | 62 | 21 | 19 | 22 | 22 |

ment concentrations among spotted mackerel of the same age among all areas, whereas most elements varied significantly between samples of different ages (Fig. 6). The samples of 3-year-old fish from Innisfail and Hervey Bay had concentrations of P that were significantly lower than those in otoliths from Bowen fish. Samples from Innisfail also had concentrations of K that were significantly lower than those from Bowen fish (HSD, $P<0.05$). No other significant difference in elemental concentrations were detected between spotted mackerel of the same age. In contrast, significant differences were found for Ba, Mg, Na, P, S, and Sr between 1- and 3-year-old fish (HSD, $P<0.05$), independent of the area from where the samples were taken, once again emphasizing the strong effect of fish age in determining the results of elemental analyses of otolith composition. Scatterplots of the elements showing significant differences, with P as the most significant and common variable, also showed differences between fish of different ages (Fig. 7). In contrast, samples of fish of the same age showed no evidence of differences in otolith composition among areas.

Principal component analysis also showed a strong separation among spotted mackerel of different ages, but no separation of groups by area of collection (Fig. 8). One-year-old and 3-year-old samples were separated on the first principal component that explained almost 50% of the total variation in the data, largely on differences in concentration of P, Sr, Ba, Na, and S. The second principal component explained another 18% of the total variation on the basis of differences in K concentration among the samples. ANOVA showed significant differences among the PC I scores ($F=318.32$, df=4, 140, $P<0.0001$), further confirming pooling of spatial samples of the same age. Bowen, Innisfail, and Hervey Bay samples of 3-year-old fish were all significantly different from Moreton Bay and Hervey Bay 1-year-old fish, whereas no differences were found among samples of the same age (HSD, $P<0.05$).

Spotted mackerel samples were best discriminated into two main groups on the basis of statistically significant differences in elemental composition of the otoliths: 1) 1-year-old fish; and 2) 3-year-old fish (Wilks's lambda 0.045) (Fig. 9). ANOVA on the CV I scores detected significant differences between the different aged samples, providing further support for these patterns ($F=472.09$, df=4, 140, $P<0.0001$). The samples of 3-year-old fish from Bowen, Innisfail, and Hervey Bay all showed significant differences from the 1-year-old fish from Moreton Bay and Hervey Bay, whereas no differences were found among samples of the same age (HSD, $P<0.05$).

Phosphorus, Sr, Na, Ba, and Mg were the main elements that caused the grouping patterns in the first discriminant function that accounted for 97% of the total variation (Table 4). One-year-old spotted mackerel tended to have higher Mg and Na concentrations and lower Ba, P, and Sr concentrations than 3-year-old fish (Fig. 6). The separation patterns observed in the individual discriminations resulted in only 57% of the spotted mackerel samples being correctly classified into their respective groups owing to the strong grouping effect of the samples into their specific age classes, rather than by area (Table 5). Pooling of the results for fish of the same age resulted in a classification success of 100% for each age class, independent of the area from where the samples were collected.

**Table 4**

Discrimination between samples of spotted mackerel determined by the pooled within-groups correlations of length-corrected elemental concentrations with the significant ($P<0.05$) canonical variates I, II, and III, and the cumulative proportion of the variance accounted for by each function, for the seven significant elements (Wilks' s lambda=0.045).

| | Canonical variate | | |
|---|---|---|---|
| Element | I | II | III |
| Ba | –0.31 | –0.45 | 0.06 |
| K | –0.06 | 0.27 | 0.64 |
| Mg | 0.26 | 0.40 | 0.23 |
| Na (length corrected) | 0.33 | –0.30 | 0.26 |
| P | –0.35 | 0.39 | 0.50 |
| S | –0.19 | –0.38 | 0.30 |
| Sr (length corrected) | –0.34 | 0.13 | –0.34 |
| Cumulative proportion | 0.97 | 0.99 | 1.00 |

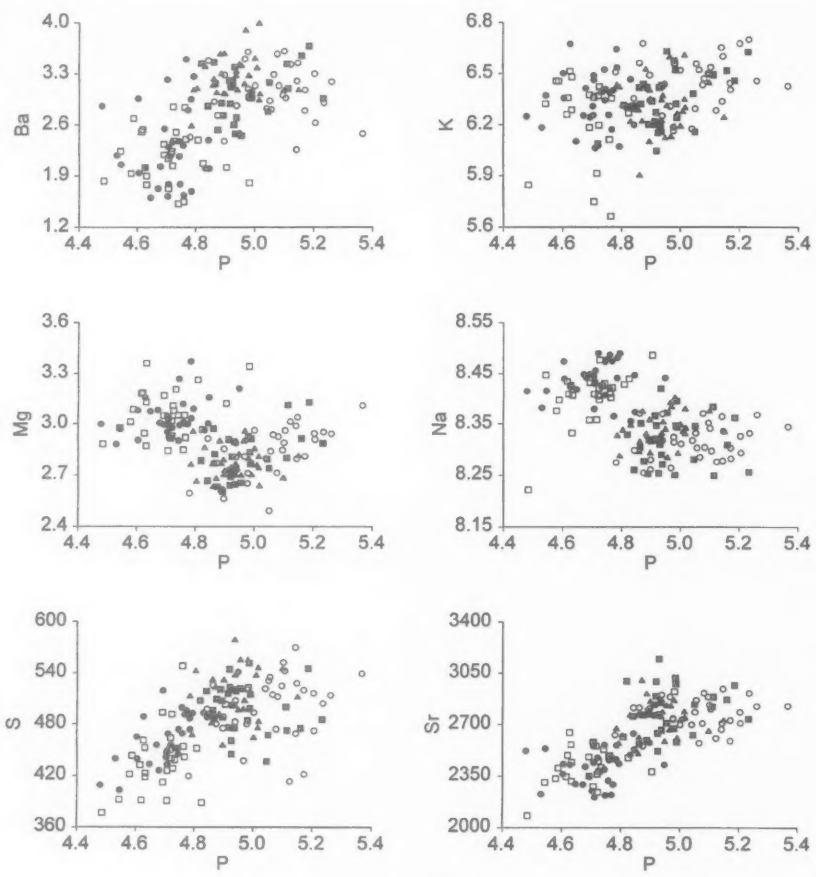


**Figure 7**

Scatterplots of significant elemental ratios showing grouping patterns of area and age spotted mackerel samples. Open circle = Bowen 3-year-old fish; open square = Hervey Bay 1-year-old fish; solid square = Hervey Bay 3-year-old fish; solid triangle = Innisfail 3-year-old fish; and solid circle = Moreton Bay 1-year-old fish. K, Mg, Na, and P are $\log_e$ transformed concentrations.

**Table 5**

Jack-knifed cross-validation classification matrix of the frequency of assigned cases in each area (and age) used to differentiate spotted mackerel samples.

| | | Classification of individual spotted mackerel by area | | | | |
|---|---|---|---|---|---|---|
| Area | Correct (%) | Bowen (3) | Hervey (1) | Hervey (3) | Innisfail (3) | Moreton (1) |
| Bowen (3) | 58 | 18 | 0 | 9 | 4 | 0 |
| Hervey (1) | 63 | 0 | 17 | 0 | 0 | 10 |
| Hervey (3) | 34 | 9 | 0 | 10 | 10 | 0 |
| Innisfail (3) | 68 | 2 | 0 | 7 | 19 | 0 |
| Moreton (1) | 63 | 0 | 11 | 0 | 0 | 19 |
| Total | 57 | 29 | 28 | 26 | 33 | 29 |

## Discussion

### Limitations of elemental analysis of whole otoliths

We found evidence of two separate groups of school mackerel and one group of spotted mackerel in the study region based on the variation in the mean elemental composition of individual fish otoliths of the same age. Such variation has been commonly presumed to reflect prolonged separation of the populations and ultimately stock divergence (Edmonds et al., 1991). However, it is not possible to infer from such studies alone what environmental, dietary, or genetic factors cause spatial patterns. Consequently, a number of wholly different but equally plausible hypotheses could be considered in determining the cause of the patterns observed in the elemental compositions of the otoliths of the two mackerel species, depending on what factors are considered important in determining otolith chemistry.

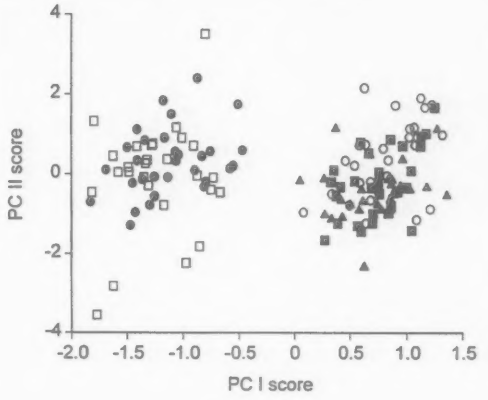


**Figure 8**

Principal component (PC) analysis of spotted mackerel otolith elemental concentrations indicating grouping patterns. Open circle = Bowen 3-year-old fish; open square = Hervey Bay 1-year-old fish; solid square = Hervey Bay 3-year-old fish; solid triangle = Innisfail 3-year- old fish; and solid circle = Moreton Bay 1-year-old fish.

An obvious explanation is that mean otolith composition differs spatially for school mackerel because their environment (or diet) varies among locations along the east coast, whereas there are no such differences for spotted mackerel because their environment (or diet) is more uniform throughout their range. For example, Proctor et al. (1995) concluded that an inability to discriminate among southern bluefin tuna (*Thunnus maccoyii*) samples with trace-element analysis was due partly to the uniformity of the pelagic environment. Water chemistry and food sources along the east coast of Queensland might be expected to vary among coastal bays, between inshore and offshore waters and between deep and shallow parts of the water column, especially given the strong influence of freshwater input in the wet-dry tropics (Thorrold and McKinnon, 1995). Indeed, the clupeid and engraulid species that form major parts of the diet of both school and spotted mackerel are generally found only in the southern half of the study area (Begg and Hopper, 1997).

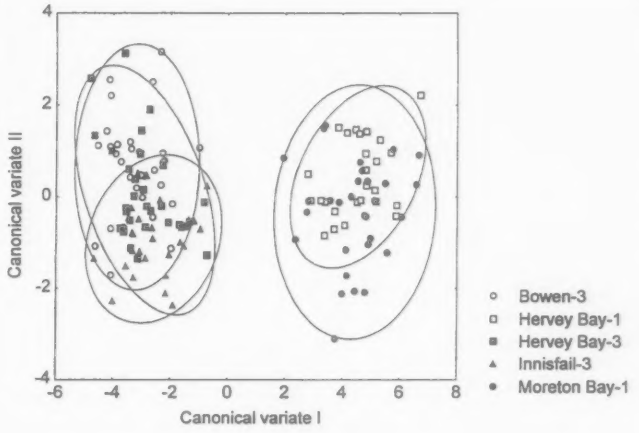


**Figure 9**

Discrimination between spotted mackerel samples based on the concentrations of the seven (length corrected) trace elements (±95% confidence ellipses around the sample mean for each area).

At present there is no fishery-independent information on the cross-shelf distribution and habitat of school and spotted mackerel and only a coarse understanding of the feeding patterns of both species. School mackerel are thought to inhabit mainly inshore waters, whereas spotted mackerel are thought to be more common offshore (Munro, 1943; Collette and Russo, 1984), although they do move to inshore waters to feed when undertaking seasonal migrations along the coastline (Begg and Hopper, 1997; Begg et al., 1997). School and spotted mackerel are often caught together in the same mixed schools during winter in the shallow northern bays

of the Queensland east coast and in summer in the southern bays, but the location and habitats of juveniles is not well known, particularly for spotted mackerel.

Complementary biological information from other stock identification methods is therefore essential to help interpret results from whole otolith analyses, but some hypotheses regarding stock structure cannot be tested in the absence of knowledge about the factors governing otolith composition. The resolution of the technique is limited further by volumetric considerations and the nature of otolith growth. Even if there were very large consistent differences in composition among individuals at the otolith core during larval and postlarval life, these would be virtually undetectable with bulk analysis, whereas relatively small differences accumulated during later life before capture would have a disproportionately large effect on mean composition. To overcome these problems—which may be accentuated for pelagic species with complex ontogenetic movements (Proctor et al., 1995)—bulk analysis of whole otoliths from juveniles, or excision and analysis of the core regions of otoliths from adult fish (Dove et al., 1996), may provide other, solution-based alternatives to sectioning and electron- or laser-probe techniques.

There may also be temporal variation in stock discrimination patterns, particularly for pelagic species living in coastal areas influenced by boundary currents; Edmonds et al. (1995) demonstrated that variation in composition of pilchard (*Sardinops sagax*) otoliths among years was significantly greater than the variation among sites. Stock discrimination patterns of pilchards were not persistent from one sampling era to the next within a decade. Further bias may be introduced by uneven representation of all life history stages within collections (Edmonds et al., 1995).

### Age-related variation

Spatial variation in the concentration of trace elements of school and spotted mackerel was strongly influenced by the age of fish in the samples at time of collection. Differential otolith elemental patterns were found between 1- and 2-year-old school mackerel, and 1- and 3-year-old spotted mackerel throughout the study region. Numerous studies, including this one, support Kalish's (1989) hypothesis that incorporation of trace elements into otoliths is related to growth (Grady et al., 1989; Thresher et al., 1994; Edmonds et al., 1995; Fowler et al., 1995). Consequently, spatial variation in elemental composition of otoliths can be difficult to interpret because of biases related to the size and age of fish in the samples from separate areas.

School and spotted mackerel of different ages from the same area have significantly different patterns in the elemental composition of their otoliths. One-year-old school and spotted mackerel tended to have lower concentrations of P, S, and Sr, while having higher levels of Mg, Mn, and Na in their otoliths compared with older fish. Grady et al. (1989) found similar results for king mackerel where heavy metal concentrations were generally higher in otoliths of younger fish. Differences in the concentrations of trace elements accumulated in the otoliths of fish of different ages from the same stock are not unexpected, particularly if irreversible deposition of elements in otoliths is assumed. Distinct chemical patterns in otoliths of fish of different ages may reflect exposure to similar environments, but for different accumulation periods, or alternatively, may reflect life history differences, such as younger fish inhabiting distinct nursery grounds that are separate from adult habitats.

Not all factors affecting deposition of elements in otoliths are strictly environmental or ontogenetic, nor do they necessarily act in a simplistic manner that reflects ambient environmental chemistry (Kalish, 1989; Campana et al., 1994). Chemical deposition can be regulated by many interacting factors, including water temperature, salinity, age, physiology, growth rates, and activity levels of individual fish (Kalish, 1989, 1990, 1991; Radtke and Shafer, 1992; Rieman et al., 1994; Fowler et al., 1995).

### Stock structure and management

Although further research is required to determine the mechanisms responsible for elemental deposition in otoliths of *Scomberomorus* species, particularly the interaction between environmental and genomic controls, the validity of using stock and site-specific elemental "fingerprints" does not rest upon the mechanism underlying otolith formation (Campana and Gagné, 1995). Optimal groupings of mean elemental composition of school mackerel otoliths strongly supported the hypothesis of at least two stocks in the study region—a hypothesis that has been developed with complementary stock identification techniques. Localized movements of tagged school mackerel have shown that there is little exchange between adult fish from different areas throughout Queensland east coast waters; most recaptures occur within the same area of release (Begg et al., 1997). Studies of the timing and location of spawning have also shown that school mackerel spawn concurrently at a number of localities along the east coast (Begg, in press). The species also exhibits differences in growth patterns and genetic variation throughout its distribution on

the east coast (Begg and Sellin, 1998; Begg et al., in press).

The limited movements of school mackerel indicated by tag-recapture data may also explain the overlap observed in the otolith composition of 2-year-old fish collected in Moreton Bay and Rockhampton. Small numbers of tagged school mackerel released in both these locations were recaptured in Hervey Bay between August and January, where they appear to be mixing on a common feeding ground (Begg et al., 1997; Begg and Hopper, 1997).

In contrast, spotted mackerel of the same year class sampled in Queensland east coast waters had similar patterns of elemental composition in their otoliths regardless of the region in which they were collected. This finding strongly supports the hypothesis of a single intermixing stock in the region of sampling derived from previous tagging, genetic, and reproductive studies, and seasonal changes in the location of commercial harvesting. These sources of information suggest an annual large-scale movement of spotted mackerel along the Queensland east coast to southern feeding grounds in summer and a return migration in winter to northern spawning grounds (Begg and Hopper, 1997; Begg et al., 1997; Begg, in press). In addition, similar growth rates and homogeneous genetic conditions of spotted mackerel throughout the study region support the hypothesis of a single east coast stock (Begg and Sellin, 1998; Begg et al., in press).

The longshore East Australian Current possibly provides cues for the migratory cycle of spotted mackerel and may facilitate larval dispersal from the spawning grounds and ultimately stock homogeneity throughout Queensland east coast waters. Proctor et al. (1995) proposed a similar stock structure for southern bluefin tuna on the basis of chemical composition of otoliths from juveniles collected along the major migration route of the species.

Identification of a species' stock structure is an important requirement for effective fisheries management (Rounsefell, 1975). The inability to define stock boundaries could unknowingly prejudice otherwise well-designed management protection efforts (Kutkuhn, 1981). Management of spotted mackerel in Queensland would be best addressed at the state level, because fishing effort in areas remote from one another may have an interaction on a single stock. In contrast, more localized (regional-level) management actions could proceed for school mackerel within Queensland—especially if the stock boundaries can be refined by further tests of the temporal persistence of the patterns described in this study.

The use of otolith trace element analysis holds great potential for stock discrimination in fisheries for other *Scomberomorus* species that share common characteristics of migration through multiple jurisdictions and fishery types. However, the use of bulk analysis of whole otoliths allows us to infer only that there has been prolonged separation of fish at some stage in their life history. Without "life history scans" across otoliths, with techniques such as electron-probe microanalysis (Gunn et al., 1992), we cannot explore the possibility that mean elemental composition is dominated by high concentrations specific to any particular life history stage. There is also need for careful selection of samples among year classes and time periods. Replication of sampling at intervals separated by several years and representation of all ontogenetic stages need to be incorporated in analyses to test for the consistency of spatial patterns in order to provide a more accurate environmental "fingerprint" for discrimination of mackerel stocks.

## Acknowledgments

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## Literature cited

**Begg, G. A.**
**In press.** Reproductive biology of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) in Queensland east-coast waters. Mar. Freshwater Res. 49.

**Begg, G. A., D. S. Cameron, and W. Sawynok.**
**1997.** Movements and stock structure of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) in Australian east-coast waters. Mar. Freshwater Res. 48:295–301.

**Begg, G. A., and G. A. Hopper.**
**1997.** Feeding patterns of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) in Queensland east-coast waters. Mar. Freshwater Res. 48: 565–571.

**Begg, G. A., C. P. Keenan, and M. J. Sellin.**
**In press.** Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. J. Fish Biol. 52.

**Begg, G. A., and M. J. Sellin.**
**1998.** Age and growth of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) in Queensland east-coast waters with implications for stock structure. Mar. Freshwater Res. 49:109–120.

**Campana, S. E., A. J. Fowler, and C. M. Jones.**
**1994.** Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 51:1942–1950.

**Campana, S. E., and J. A. Gagné.**
**1995.** Cod stock discrimination using ICPMS elemental assays of otoliths. *In* D. H. Secor, J. M. Dean, and S. E. Campana (eds.), Recent developments in fish otolith research, p. 671–691. Univ. South Carolina Press, Columbia, SC.

**Campana, S. E., J. A. Gagné, and J. W. McLaren.**
**1995.** Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. 122: 15–120.

**Collette, B. B., and J. L. Russo.**
**1984.** Morphology, systematics, and biology of the Spanish mackerels (*Scomberomorus*, Scombridae). Fish. Bull. 82:545–689.

**Dove, S. G., B. M. Gillanders, and M. J. Kingsford.**
**1996.** An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fishes. J. Exp. Mar. Biol. Ecol. 205:15–34.

**Edmonds, J. S., N. Caputi, and M. Morita.**
**1991.** Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Aust. J. Mar. Freshwater Res. 42:383–389.

**Edmonds, J. S., N. Caputi, M. J. Moran, W. J. Fletcher, and M. Morita.**
**1995.** Population discrimination by variation in concentrations of minor and trace elements in sagittae of two Western Australian teleosts. *In* D. H. Secor, J. M. Dean, and S. E. Campana (eds.), Recent developments in fish otolith research, p. 655–670. Univ. South Carolina Press, Columbia, SC.

**Edmonds, J. S., M. J. Moran, N. Caputi, and M. Morita.**
**1989.** Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. Can. J. Fish. Aquat. Sci. 46:50–54.

**Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold.**
**1995.** Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 52:1431–1441.

**Grady, J. R., A. G. Johnson, and M. Sanders.**
**1989.** Heavy metal content in otoliths of king mackerel (*Scomberomorus cavalla*) in relation to body length and age. Contrib. Mar. Sci. 31:17–23.

**Gunn, J. S., I. R. Harrowfield, C. H. Proctor, and R. E. Thresher.**
**1992.** Electron probe microanalysis of fish otoliths—evaluation of techniques for studying age and stock discrimination. J. Exp. Mar. Biol. Ecol. 158:1–36.

**Ihssen, P. E., H. E. Booke, J. M. Casselman, J. M. McGlade, N. R. Payne, and F. M. Utter.**
**1981.** Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38:1838–1855.

**Johnson, A. G., W. A. Fable Jr., C. B. Grimes, and L. Trent.**
**1994.** Evidence for distinct stocks of king mackerel, *Scomberomorus cavalla*, in the Gulf of Mexico. Fish. Bull. 92:91–101.

**Kalish, J. M.**
**1989.** Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132:151–178.
**1990.** Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish. Bull. 88:657–666.
**1991.** Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. Mar. Ecol. Prog. Ser. 74:137–159.

**Kalish, J. M., M. E. Livingston, and K. A. Schofield.**
**1996.** Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. Mar. Freshwater Res. 47:537–542.

**Kutkuhn, J. H.**
**1981.** Stock definition as a necessary basis for cooperative management of Great Lakes fish resources. Can. J. Fish. Aquat. Sci. 38:1476–1478.

**Mulligan, T. J., F. D. Martin, R. A. Smucker, and D. A. Wright.**
**1987.** A method of stock identification based on the elemental composition of striped bass *Morone saxatilis* (Walbaum) otoliths. J. Exp. Mar. Biol. Ecol. 114:241–248.

**Munro, I. S. R.**
**1943.** Revision of Australian species of *Scomberomorus*. Mem. Qld. Mus.12:65–95.

**Proctor, C. H., R. E. Thresher, J. S. Gunn, D. J. Mills, I. R. Harrowfield, and S. H. Sie.**
**1995.** Stock structure of the southern bluefin tuna *Thunnus maccoyii*: an investigation based on probe microanalysis of otolith composition. Mar. Biol. 122:511–526.

**Radtke, R. L., and D. J. Shafer.**
**1992.** Environmental sensitivity of fish otolith microchemistry. Aust. J. Mar. Freshwater Res. 43:935–951.

**Rieman, B. E., D. L. Myers, and R. L. Neilsen.**
**1994.** Use of otolith microchemistry to discriminate *Oncorhyncus nerka* of resident and anadromous origin. Can. J. Fish. Aquat. Sci. 51:68–77.

**Rounsefell, G. A.**
**1975.** Ecology, utilization, and management of marine fisheries. Mosby, St. Louis, MO, 516 p.

**Shaklee, J. B., S. R. Phelps, and J. Salini.**
**1990.** Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterization of allelic isozymes. *In* D. H. Whitmore (ed.), Electrophoretic and isoelectric focusing techniques in fisheries management, p. 173–196. CRC Press, Boca Raton. FL.

**Sutter, F. C., III, R. O. Williams, and M. F. Godcharles.**
**1991.** Movement patterns and stock affinities of king mackerel in the southeastern United States. Fish. Bull. 89:315–324.

**SYSTAT.**
**1997.** SYSTAT 7.0 statistics. SPSS Incorporated, Chicago, IL, 751 p.

**Thorrold, S. R., and A. D. McKinnon.**
**1995.** Response of larval fish assemblages to a riverine plume in coastal waters of the central Great Barrier Reef lagoon. Limnol. Oceanogr. 40:177–181.

**Thresher, R. E., C. H. Proctor, J. S. Gunn, and I. R. Harrowfield.**
**1994.** An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). Fish. Bull. 92:817–840.

**Trent, L., B. J. Palko, M. L. Williams, and H. A. Brusher.**
**1987.** Abundance of king mackerel, *Scomberomorus cavalla*, in the southeastern United States based on CPUE data from charterboats 1982–85. Mar. Fish. Rev. 49:78–90.

# Pelagic sharks associated with the swordfish, *Xiphias gladius*, fishery in the eastern North Atlantic Ocean and the Strait of Gibraltar

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**Abstract.**—We report on 175 landings from 106 longline and 69 gillnet boats operating in the eastern North Atlantic Ocean and Mediterranean Sea, July 1991 to July 1992. Information on the catch and biology of five shark species (*Isurus oxyrinchus*, *Prionace glauca*, *Alopias superciliosus*, *Alopias vulpinus*, and *Sphyrna zygaena*) is analyzed and contrasted with swordfish (*Xiphias gladius*) landings. A total of 51,205 fish were sampled, of which 40,198 were sharks, 9,990 swordfish, and the rest other bony fish. Spatial, temporal, and gear analyses were performed to show the importance of shark bycatch in longline and gillnet fisheries operating from the south of Spain. We present information on population structures of the shark species, along with hypotheses about shortfin mako movements as suggested by landing data.

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Fish. Bull. 96:667–685 (1998).

The Spanish longline fleet operates in the eastern North Atlantic (FAO fishing area 27), north-central Atlantic (FAO 34), and the Mediterranean Sea (FAO 37). Sharks are the most important bycatch of swordfish (*Xiphias gladius* Linnaeus, 1758) fishing in all of these areas (Moreno and Morón, 1992b; Mejuto and Garcés[1]; Mejuto[2]). The fleets use surface longlines to catch swordfish and some sharks have become target species as well.

Three types of longliners operate along the south Spanish coast: "coastal longliners" that fish two to five days in the area of the Strait of Gibraltar and that in some cases use gill nets in spring and summer; "offshore longliners" that spend 15 to 25 days fishing as far south as Senegal; and "long distance freezer longliners" that operate mainly in the tropical Atlantic Ocean (Gulf of Guinea and off Brazil). Little is known about shark fishing off southern Spain (García, 1970; Bravo and Santaella, 1973; Amorín et al., 1979; Garcés and Rey, 1983; Moreno and Morón, 1992b); more is known about shark catches from the northern Spanish longline fleet (Mejuto and Garcés[1]; Mejuto[2]).

The biology of eastern Atlantic pelagic sharks has not been well investigated although detailed taxonomic studies (Moreno, 1982), morphology and growth studies (Blasco and Muñoz-Chápuli, 1981; Muñoz-Chápuli and Blasco, 1984; Moreno and Morón, 1992b), reproduction (Muñoz-Chápuli, 1984; Moreno et al., 1989; Moreno and Morón, 1992a), and faunistic studies (Belloc, 1934; Lozano Cabo, 1950; Bravo, 1974; Bravo[3]) are available.

The most common shark species taken by longliners are blue shark, *Prionace glauca* (Linnaeus, 1758) and shortfin mako shark, *Isurus oxyrinchus* Rafinesque, 1810, which represent 80% of the total shark bycatch (Garcés and Rey, 1983; Moreno and Morón, 1992b). Other sharks caught include the porbeagle, *Lamna nasus* (Bonnaterre, 1788), the hammerheads, *Sphyrna* spp., the bigeye thresher, *Alopias superciliosus* (Lowe, 1839), the common thresher, *Alopias vulpinus*

[1] Mejuto, J., and A. Garcés. 1984. Shortfin mako, *Isurus oxyrinchus*, and porbeagle, *Lamna nasus*, associated with longline swordfish fishery in NW and N Spain. ICES, Council Meeting 1984/G: 72.

[2] Mejuto, J. 1985 Associated catches of sharks, *Prionace glauca*, *Isurus oxyrinchus* and *Lamna nasus*, with NW and N Spanish swordfish fishery, in 1984. ICES, Council Meeting 1985/H: 42.

[3] Bravo, J. 1973. Elasmobranchii off Canary Islands. ICES, Council Meeting 1973/J: 17.

(Bonnaterre, 1788), and the requiem sharks, *Carcharhinus* spp. (Garcés and Rey, 1984). Bony fish are caught occasionally, including the northern bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758), bigeye tuna, *Thunnus obesus* (Lowe, 1839), albacore tuna, *Thunnus alalunga* (Bonnaterre, 1788), oilfish, *Ruvettus pretiosus* Cocco, 1829, escolar, *Lepidocybium flavobrunneum* (Smith, 1849), and longbill spearfish, *Tetrapturus pfluegeri* Robins and de Sylva, 1963, (Mejuto and Garcés[1]; Mejuto[2]).

## Materials and methods

Between July 1991 and July 1992, 106 longline and 69 gillnet landings (85.4% and 87.3% respectively of total longline and gillnet landings) were sampled at the Algeciras fish market (Cádiz, southern Spain), the largest market in southwestern Spain receiving 80% of the longline catch from this area (Anonymous, 1986). Data on the number of fish landed by species, days of each trip, active fishing days, number of sets and hooks for longliners, and net length and fishing time for gillnetters were collected during interviews with skippers.

The longline catch rate was calculated as hook rate (HR=number of fish/1,000 hooks), and gillnet catch rate was calculated as the average net length by trip multiplied by the number of sets. No total catch and effort data were available; catch rates are approximate indicators and do not reflect actual abundance. Fishing time was not included in effort calculations because it was constant for every boat and gear. The gears were set at dusk and retrieved before sunrise. Longlines ranged from 18 to 29 km in length and on average had 1500 hooks (range: 475 to 2500), set at a depth of 11 to 55 m. Gill nets were 2.5 km long, 14 m high, and had a mesh size of 40 cm.

All fish landed were identified to species level and counted. Most sharks were sexed and measured to the nearest centimeter for total length (TL) or fork length (FL), or both. Total length was measured from the rear tip of the upper caudal lobe to the snout tip, along the horizontal line of the body axis. Length data were grouped in 5-cm intervals of FL (shortfin mako and blue sharks) and TL (thresher and hammerhead sharks) for length-frequency analysis. Biometrical relationships between sexes were performed by simple linear regression analysis when the sample size was sufficiently large (shortfin mako, blue, and bigeye thresher sharks). Standard length (SL) has been used as the reference length for the relation with fork length (FL), total length (TL), and upper caudal lobe (UCL) (dorsal-caudal margin). The relation between FL and TL was also calculated. The area sampled (Fig. 1) was divided into 5° latitude sectors from 20°N to 40°N. Sectors 4 and 5 have the same latitude (35°N–40°N) but different longitudes, 3°W–8°W for sector 5, 8°W–13°W for sector 4. This division separates the gillnet fishing ground (sector 5) from the longline Atlantic area (sector 4).

A chi-square ($\chi^2$) test was performed to test the fit of the sample to a normal distribution and to observe the variations in species by sector and month. Strong bias was expected because of the effect of discarded fish at sea. Single factor ANOVA was performed to compare the means of the samples by area.

## Results

A total of 40,198 sharks and 11,007 bony fish were sampled from 175 landings from July 1991 to July 1992 (Table 1). The proportion of sharks landed was higher with longlines than with gill nets, but this might be related to the seasonal abundance of sharks and swordfish in the Strait of Gibraltar during the period when gill nets were used (Fig. 2).

The most common species landed by longlines in the areas studied were blue shark, shortfin mako shark, and swordfish (Table 2). Other chondrichthyans were less common, some rarely occurring in more than 10% of the landings.

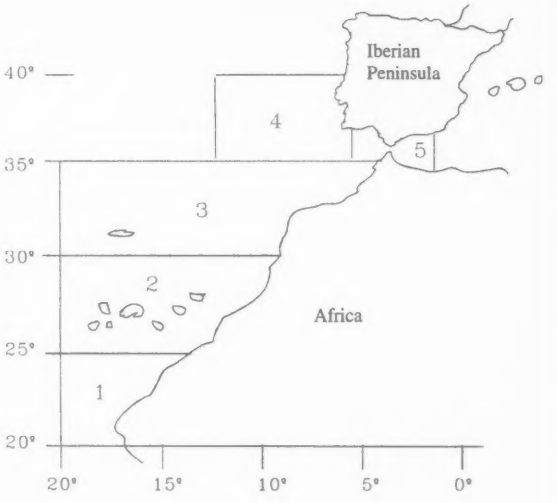


**Figure 1**

Map of the study area showing the five sectors into which the area was divided for the present paper.

Proportions of swordfish and the five most common shark species landed by longlines were significantly different ($\chi^2$=5.41; df=20; $P$<0.001) in each sector (Table 3). The highest proportion of fish in all the sectors, except one (sector 1), was that of blue sharks, whereas the lowest proportion in all five sectors was that of the common thresher shark. Swordfish occurred with decreasing proportion from south to north in the Atlantic sectors, reaching a minimum in the Mediterranean sector. Shortfin mako sharks steadily decreased from sector 1 to sector 4. The proportion of sharks, overall, was greater than the proportion of swordfish in all sectors, and sharks were included in at least 80% of the landings in all sectors, where they were slightly greater in numbers than swordfish, except in sector 1.

**Table 1**

Total number of fish sampled from 175 landings at the Algeciras (Cádiz) fish market and percentage by species from July 1991 to July 1992.

| Species | Number | Percentage |
|---|---|---|
| *Isurus oxyrinchus* | 5947 | 11.6 |
| *Prionace glauca* | 32,661 | 63.7 |
| *Alopias vulpinus* | 52 | 0.1 |
| *Alopias superciliosus* | 557 | 1.1 |
| *Sphyrna zygaena* | 757 | 1.4 |
| Other sharks | 224 | 0.4 |
| Total sharks | 40,198 | 78.5 |
| *Xiphias gladius* | 9990 | 19.5 |
| Other bony fish | 1017 | 2.0 |
| Total | 51,205 | 100.0 |

The proportional distribution of fish from gillnet landings was significantly different from that of fish from longline landings in the same sector ($\chi^2$=2.97; df=5; $P$<0.001) (Table 3). Gill nets were operated only in sector 5. Swordfish represented a much higher proportion in total gillnet landings than in longline landings; sharks represented only one third of total gillnet landings. The drop in the relative importance of sharks was due mainly to a much lower number of blue sharks in gillnet landings than in longline landings (8% compared with 82%). Bigeye thresher sharks slightly increased in landings (from 0.01% to 6%).

The proportions of fish landed by longline differed significantly by month ($\chi^2$=3.55; df=48; $P$<0.001) (*A. vulpinus* not considered) (Table 4), although blue shark was always the most predominant. Swordfish and shortfin mako sharks had the next highest proportions, whereas the other species never reached more than 5% of monthly catches. Sharks always accounted for more than 50% of the monthly catch, and more than 80% in 10 out the 13 months sampled.

Proportions of fish in gillnet landings also differed significantly by month ($\chi^2$=1.10; df=35; $P$<0.001) (March not considered) (Table 4). Swordfish had the highest proportion, except during the month of April. Blue sharks were taken in small proportions for most months (0–37%). The proportion of thresher sharks varied from month to month. Bigeye thresher sharks were taken at a maximum (10%) in August 1991 and July 1992; common thresher sharks also reached 10% in January 1992. The proportion of sharks exceeded

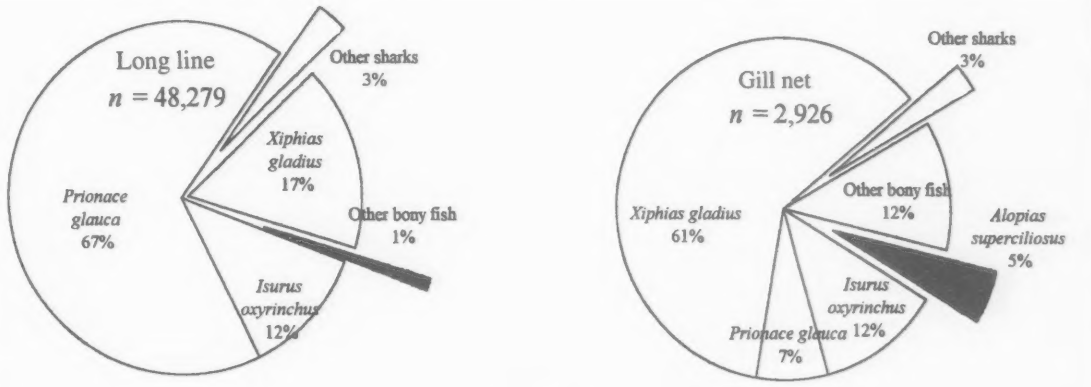


**Figure 2**

Species composition (% of fish) landed by the gillnet and longline fisheries operating in the eastern Atlantic Ocean and western Mediterranean Sea.

**Table 2**

Species occurrence by number of landings and proportion (%) by gear.

| Species | Appearance (no. of landings) | | | Percentage | | |
|---|---|---|---|---|---|---|
| | Longline | Gillnet | Total | Longline | Gillnet | Total |
| *Isurus oxyrinchus* Rafinesque, 1810 | 102 | 45 | 147 | 96.2 | 65.2 | 84.0 |
| *Isurus paucus* Guitart Manday, 1966 | 11 | 1 | 12 | 10.4 | 1.4 | 6.8 |
| *Lamna nasus* (Bonnaterre, 1788) | 2 | 5 | 7 | 1.9 | 7.2 | 4.0 |
| *Alopias superciliosus* (Lowe, 1839) | 61 | 23 | 84 | 57.5 | 33.3 | 48.0 |
| *Alopias vulpinus* (Bonnaterre, 1788) | 11 | 11 | 22 | 10.4 | 15.9 | 12.6 |
| *Carcharhinus* spp. | 7 | 0 | 7 | 6.6 | 0 | 4.0 |
| *Carcharhinus falciformis* (Bibron, 1841) | 2 | 0 | 2 | 1.9 | 0 | 1.1 |
| *Galeocerdo cuvier* (Péron and Lesueur, 1822) | 1 | 0 | 1 | 0.9 | 0 | 0.6 |
| *Prionace glauca* (Linnaeus, 1758) | 102 | 19 | 121 | 96.2 | 27.5 | 69.1 |
| *Sphyrna zygaena* (Linnaeus, 1758) | 43 | 26 | 69 | 40.6 | 37.7 | 39.4 |
| *Mobula mobular* (Bonnaterre, 1788) | 2 | 0 | 2 | 1.9 | 0 | 1.1 |
| *Polyprion americanus* (Schneider, 1801) | 2 | 0 | 2 | 1.9 | 0 | 1.1 |
| *Coryphaena hippurus* Linnaeus, 1758 | 3 | 7 | 10 | 2.8 | 10.1 | 5.7 |
| *Brama brama* (Bonnaterre, 1788) | 0 | 1 | 1 | 0 | 1.4 | 0.6 |
| *Sparus pagrus* Linnaeus, 1758 | 1 | 0 | 1 | 0.9 | 0 | 0.6 |
| *Lepidocybium flavobrunneum* (Smith, 1849) | 37 | 1 | 38 | 34.9 | 1.4 | 21.7 |
| *Ruvettus pretiosus* Cocco, 1829 | 5 | 0 | 5 | 4.7 | 0 | 2.8 |
| *Acanthocybium solandri* (Cuvier, 1832) | 1 | 1 | 2 | 0.9 | 1.4 | 1.1 |
| *Auxis* spp. | 0 | 4 | 4 | 0 | 5.8 | 2.3 |
| *Auxis rochei* (Risso, 1810) | 0 | 1 | 1 | 0 | 1.4 | 0.6 |
| *Katsuwonus pelamis* (Linnaeus, 1758) | 1 | 2 | 3 | 0.9 | 2.9 | 1.7 |
| *Thunnus alalunga* (Bonnaterre, 1788) | 6 | 3 | 9 | 5.7 | 4.3 | 5.1 |
| *Thunnus albacares* (Bonnaterre, 1788) | 4 | 0 | 4 | 3.8 | 0 | 2.3 |
| *Thunnus obesus* (Lowe, 1839) | 16 | 1 | 17 | 15.1 | 1.4 | 9.7 |
| *Thunnus thynnus* (Linnaeus, 1758) | 4 | 3 | 7 | 3.8 | 4.3 | 4.0 |
| *Makaira nigricans* Lacépède, 1801 | 0 | 4 | 4 | 0 | 5.8 | 2.3 |
| *Tetrapturus* spp. | 14 | 21 | 35 | 13.2 | 30.4 | 20.0 |
| *Tetrapturus albidus* Poey, 1860 | 1 | 2 | 3 | 0.9 | 2.9 | 1.7 |
| *Xiphias gladius* Linnaeus, 1758 | 101 | 63 | 164 | 95.3 | 91.3 | 93.7 |
| Total landings | 106 | 69 | 175 | 60.6 | 39.4 | 100.0 |

**Table 3**

Total number of the five most important shark species and swordfish recorded in landings and percentage by species, gear, and sector. Sectors (1–5) are indicated under gear type.

| | Number | | | | | | Percentage | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Longline | | | | | Gillnet | Longline | | | | | Gillnet |
| | 1 | 2 | 3 | 4 | 5 | 5 | 1 | 2 | 3 | 4 | 5 | 5 |
| *I. oxyrinchus* | 915 | 1648 | 2211 | 578 | 241 | 354 | 17 | 14 | 12 | 7 | 10 | 14 |
| *P. glauca* | 1865 | 7757 | 14207 | 6538 | 2082 | 212 | 34 | 64 | 76 | 76 | 82 | 8 |
| *A. vulpinus* | 4 | 1 | 7 | 2 | 2 | 36 | 0 | 0 | 0 | 0 | 0 | 1 |
| *A. superciliosus* | 28 | 266 | 101 | 7 | 9 | 146 | 1 | 2 | 1 | 0 | 0 | 6 |
| *S. zygaena* | 188 | 39 | 392 | 82 | 2 | 54 | 3 | 0 | 2 | 1 | 0 | 2 |
| Total sharks | 3000 | 9711 | 16918 | 7207 | 2336 | 802 | 55 | 80 | 90 | 84 | 92 | 31 |
| *X. gladius* | 2410 | 2426 | 1822 | 1386 | 199 | 1747 | 45 | 20 | 10 | 16 | 8 | 69 |
| Total | 5410 | 12137 | 18740 | 8593 | 2535 | 2549 | 11 | 24 | 38 | 17 | 5 | 5 |

swordfish in only one of nine months sampled for gillnet landings.

### Shortfin mako shark

Longline catch rates are shown in Table 5; Atlantic catch rates were always higher than those in the Mediterranean. Variation in the longline catch rate (Table 6) was at a minimum in December and May, peaking in April and September. In gillnet fishing (Table 7), shortfin mako shark catch rates were relatively lower than swordfish catch rates.

Table 8 gives biometrical information by sex, and longline and gillnet frequency distributions of different lengths by sex are presented in Figures 3 and 4. The distribution was not significantly different between sexes in longline ($\chi^2$=54.4; df=39; $P$>0.05) and gillnet ($\chi^2$=20.5; df=23; $P$>0.5) landings. The distribution by size of males varied significantly by sector ($F$=28.796; df=5, 1428; $P$<0.001). The distribution by size of females also varied significantly by sector ($F$=14.985; df=5, 1429; $P$<0.001).

The maximum size of females landed by longlines tended to increase from sector 1 to sector 3 (Fig. 5A). The maximum size of males tended to increase in Atlantic sectors from south to north (Fig. 5B). Smallest maximum sizes for both sexes occurred in the Mediterranean Sea, and in sector 1 for males. Gill nets catch bigger females and smaller males than do longlines in the same sector. The monthly variations

**Table 4**

Monthly longline and gillnet catch distribution for the five most important shark species and for swordfish by number of fish ($n$) and percentage (%). Species codes: IO = *Isurus oxyrinchus*; PG = *Prionace glauca*; AV = *Alopias vulpinus*; AS = *Alopias superciliosus*; SZ = *Sphyrna zygaena*; TOT-SHX = Total sharks; XG = *Xiphias gladius*;

| Gear and month | IO | | PG | | AV | | AS | | SZ | | TOT-SHX | | XG | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* |
| Longline | | | | | | | | | | | | | | | |
| Jul 91 | 224 | 16.85 | 992 | 74.64 | 2 | 0.15 | 31 | 2.33 | 21 | 1.58 | 1270 | 95.56 | 59 | 4.44 | 1329 |
| Aug 91 | 891 | 19.96 | 2865 | 64.19 | 1 | 0.02 | 3 | 0.07 | 147 | 3.29 | 3907 | 87.54 | 556 | 12.46 | 4463 |
| Sep 91 | 422 | 15.93 | 2158 | 81.46 | 0 | 0 | 7 | 0.26 | 28 | 1.06 | 2615 | 98.72 | 34 | 1.28 | 2649 |
| Oct 91 | 584 | 9.57 | 3338 | 54.68 | 0 | 0 | 32 | 0.52 | 131 | 2.15 | 4085 | 66.91 | 2020 | 33.09 | 6105 |
| Nov 91 | 970 | 14.10 | 4333 | 62.99 | 4 | 0.06 | 25 | 0.36 | 252 | 3.66 | 5584 | 81.17 | 1295 | 18.83 | 6879 |
| Dec 91 | 30 | 12.35 | 105 | 43.21 | 0 | 0 | 5 | 2.06 | 0 | 0 | 140 | 57.61 | 103 | 42.39 | 243 |
| Jan 92 | 863 | 10.02 | 6117 | 71.03 | 4 | 0.05 | 110 | 1.28 | 30 | 0.35 | 7124 | 82.72 | 1488 | 17.28 | 8612 |
| Feb 92 | 868 | 9.07 | 6988 | 73.03 | 2 | 0.02 | 135 | 1.41 | 79 | 0.83 | 8072 | 84.36 | 1497 | 15.64 | 9569 |
| Mar 92 | 423 | 9.04 | 3208 | 68.53 | 1 | 0.02 | 54 | 1.15 | 1 | 0.02 | 3687 | 78.77 | 994 | 21.23 | 4681 |
| Apr 92 | 109 | 10.64 | 913 | 89.16 | 0 | 0 | 1 | 0.10 | 0 | 0 | 1023 | 99.90 | 1 | 0.10 | 1024 |
| May 92 | 25 | 8.01 | 283 | 90.71 | 1 | 0.32 | 0 | 0 | 0 | 0 | 309 | 99.04 | 3 | 0.96 | 312 |
| Jun 92 | 114 | 10.45 | 783 | 71.77 | 1 | 0.09 | 8 | 0.73 | 2 | 0.18 | 908 | 83.23 | 183 | 16.77 | 1091 |
| Jul 92 | 70 | 15.28 | 366 | 79.91 | 0 | 0 | 0 | 0 | 12 | 2.62 | 448 | 97.82 | 10 | 2.18 | 458 |
| Total | 5593 | 11.80 | 32449 | 68.44 | 16 | 0.03 | 411 | 0.87 | 703 | 1.48 | 39172 | 82.62 | 8243 | 17.38 | 47415 |

| Gear and month | IO | | PG | | AV | | AS | | SZ | | TOT-SHX | | XG | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* | % | *n* |
| Gillnet | | | | | | | | | | | | | | | |
| Jul 91 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Aug 91 | 19 | 7.39 | 0 | 0 | 1 | 0.39 | 37 | 14.40 | 5 | 1.95 | 62 | 24.12 | 195 | 75.88 | 257 |
| Sep 91 | 106 | 22.70 | 30 | 6.42 | 2 | 0.43 | 27 | 5.78 | 22 | 4.71 | 187 | 40.04 | 280 | 59.96 | 467 |
| Oct 91 | 53 | 9.62 | 2 | 0.36 | 0 | 0 | 3 | 0.54 | 12 | 2.18 | 70 | 12.70 | 481 | 87.30 | 551 |
| Nov 91 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Dec 91 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Jan 92 | 6 | 6.25 | 0 | 0 | 15 | 15.6 | 0 | 0 | 1 | 1.04 | 22 | 22.92 | 74 | 77.08 | 96 |
| Feb 92 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Mar 92 | 0 | 0 | 1 | 33.33 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 33.33 | 2 | 66.67 | 3 |
| Apr 92 | 54 | 24.00 | 83 | 36.89 | 15 | 6.67 | 7 | 3.11 | 2 | 0.89 | 161 | 71.56 | 64 | 28.44 | 225 |
| May 92 | 76 | 11.66 | 90 | 13.80 | 1 | 0.15 | 0 | 0 | 0 | 0 | 167 | 25.61 | 485 | 74.39 | 652 |
| Jun 92 | 14 | 15.22 | 1 | 1.09 | 0 | 0 | 6 | 6.52 | 4 | 4.35 | 25 | 27.17 | 67 | 72.83 | 92 |
| Jul 92 | 26 | 12.62 | 5 | 2.43 | 2 | 0.97 | 66 | 32.04 | 8 | 3.88 | 107 | 51.94 | 99 | 48.06 | 206 |
| Total | 354 | 13.89 | 212 | 8.32 | 36 | 1.41 | 146 | 5.73 | 54 | 2.12 | 802 | 31.46 | 1747 | 68.54 | 2549 |

**Table 5**

Longline effort (number of hooks × 1000) and catch rates (number of fish/1000 hooks) by species and sector.

| Sector | Effort | *I. oxyrinchus* | *P. glauca* | *A. vulpinus* | *A. superciliosus* | *S. zygaena* | Total sharks | *X. gladius* |
|---|---|---|---|---|---|---|---|---|
| 1 | 302.4 | 3.03 | 6.17 | 0.013 | 0.09 | 0.62 | 9.92 | 7.97 |
| 2 | 534.0 | 3.09 | 14.53 | 0.002 | 0.50 | 0.07 | 18.19 | 4.54 |
| 3 | 462.9 | 4.78 | 30.69 | 0.015 | 0.22 | 0.85 | 36.55 | 3.94 |
| 4 | 61.8 | 3.90 | 33.69 | 0.032 | 0.15 | 0.03 | 37.80 | 3.22 |
| 5 | 297.5 | 1.94 | 21.98 | 0.007 | 0.02 | 0.28 | 24.23 | 4.66 |
| Total | 1658.6 | 3.37 | 19.56 | 0.010 | 0.25 | 0.42 | 23.62 | 4.97 |

**Table 6**

Longline effort (number of hooks × 1000) and catch rates (number of fish/1000 hooks) by species and month.

| Month | Effort | *I. oxyrinchus* | *P. glauca* | *A. vulpinus* | *A. superciliosus* | *S. zygaena* | Total sharks | *X. gladius* |
|---|---|---|---|---|---|---|---|---|
| Jul 91 | 67.40 | 3.32 | 14.72 | 0.03 | 0.46 | 0.31 | 18.84 | 0.88 |
| Aug 91 | 180.40 | 4.94 | 15.88 | 0.01 | 0.02 | 0.81 | 21.66 | 3.08 |
| Sep 91 | 41.30 | 10.22 | 52.25 | 0.00 | 0.17 | 0.68 | 63.32 | 0.82 |
| Oct 91 | 195.00 | 2.99 | 17.12 | 0.00 | 0.16 | 0.67 | 20.95 | 10.36 |
| Nov 91 | 185.20 | 5.24 | 23.40 | 0.02 | 0.13 | 1.36 | 30.15 | 6.99 |
| Dec 91 | 16.80 | 1.79 | 6.25 | 0.00 | 0.30 | 0.00 | 8.33 | 6.13 |
| Jan 92 | 296.60 | 2.91 | 20.62 | 0.01 | 0.37 | 0.10 | 24.02 | 5.02 |
| Feb 92 | 393.20 | 2.21 | 17.77 | 0.01 | 0.34 | 0.20 | 20.53 | 3.81 |
| Mar 92 | 206.90 | 2.04 | 15.51 | 0.00 | 0.26 | 0.00 | 17.82 | 4.80 |
| Apr 92 | 5.00 | 21.80 | 182.60 | 0.00 | 0.20 | 0.00 | 204.60 | 0.20 |
| May 92 | 12.80 | 1.95 | 22.11 | 0.08 | 0.00 | 0.00 | 24.14 | 0.23 |
| Jun 92 | 47.00 | 2.43 | 16.66 | 0.02 | 0.17 | 0.04 | 19.32 | 3.89 |
| Jul 92 | 11.00 | 6.36 | 33.27 | 0.00 | 0.00 | 1.09 | 40.73 | 0.91 |
| Total | 1,658.60 | 3.37 | 19.56 | 0.01 | 0.25 | 0.42 | 23.62 | 4.97 |

**Table 7**

Gillnet effort (average net length (number of sets) and catch rates (number of fish/unit of effort) by species and month.

| Sector | Effort | *I. oxyrinchus* | *P. glauca* | *A. vulpinus* | *A. superciliosus* | *S. zygaena* | Total sharks | *X. gladius* |
|---|---|---|---|---|---|---|---|---|
| Jul 91 | — | — | — | — | — | — | — | — |
| Aug 91 | 118.50 | 0.16 | 0.00 | 0.01 | 0.31 | 0.04 | 0.52 | 1.65 |
| Sep 91 | 152.90 | 0.69 | 0.20 | 0.01 | 0.18 | 0.14 | 1.22 | 1.83 |
| Oct 91 | 90.90 | 0.58 | 0.02 | 0.00 | 0.03 | 0.13 | 0.77 | 5.29 |
| Nov 91 | — | — | — | — | — | — | — | — |
| Dec 91 | — | — | — | — | — | — | — | — |
| Jan 92 | 36.50 | 0.16 | 0.00 | 0.41 | 0.00 | 0.03 | 0.60 | 2.03 |
| Feb 92 | — | — | — | — | — | — | — | — |
| Mar 92 | 2.00 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 0.50 | 1.00 |
| Apr 92 | 48.00 | 1.13 | 1.73 | 0.31 | 0.15 | 0.04 | 3.35 | 1.33 |
| May 92 | 122.50 | 0.62 | 0.73 | 0.01 | 0.00 | 0.00 | 1.36 | 3.96 |
| Jun 92 | 30.00 | 0.47 | 0.03 | 0.00 | 0.20 | 0.13 | 0.83 | 2.23 |
| Jul 92 | 56.00 | 0.46 | 0.09 | 0.04 | 1.18 | 0.14 | 1.91 | 1.77 |
| Total | 657.30 | 0.54 | 0.32 | 0.05 | 0.22 | 0.08 | 1.22 | 2.66 |

of maximum, median, and minimum sizes are presented in Figure 6, A and B.

### Blue shark

Blue sharks are often discarded at sea because they are not commercially valuable in the Spanish market; real catch rates are biased by this factor, especially in the more distant sectors where the tendency is to try to improve long trips with more valuable species, i.e. with swordfish and shortfin mako shark. Therefore, sectors 1 and 2 had lower catch rates, based on landings, than sectors 3, 4, and 5 (Table 5). Longline catch rates by month (Table 6) ranged from 6 to 52 fish/1000 hooks, except for an extremely high rate in April (182 fish/1000 hooks). Gillnet catch rates for this species were very low in relation to other species, in some months almost negligible (Table 7).

In the longline fishery, sex ratio by sector was close to 1 male:0.25 females, except in sector 3 where it was almost equal (1 male:0.92 females); gillnet sex ratio (1 male:2.45 females) was inverse to that observed for longlines in the same sector. Monthly data were not sufficient to be analyzed and therefore were grouped by quarter, i.e. July–September 1991, October–December 1991, January–March 1992, and April–June 1992. Because of the effect of discarded fish at sea and inappropriate sex sampling, we were unable to make significant assertations about sex ratio variability.

The distribution of overall length frequency is presented in Figure 7A. In longline landings, modal

**Table 8**

Biometric relations of standard length (*SL*) with fork length (*FL*), total length (*TL*), and upper caudal lobe length (UCL); and of fork length with total length by sex for *Isurus oxyrinchus*, *Prionace glauca*, and *Alopias superciliosus*, and both sexes combined for *Alopias vulpinus* and *Sphyrna zygaena*.

| Females | Males | Total |
|---|---|---|
| *Isurus oxyrinchus* | | |
| $FL = 1.086\ SL + 1.630$ | $FL = 1.086\ SL + 1.409$ | $FL = 1.086\ SL + 1.515$ |
| $r^2 = 0.993\ n = 852$ | $r^2 = 0.993\ n = 911$ | $r^2 = 0.993\ n = 1{,}763$ |
| $TL = 0.817\ SL + 0.400$ | $TL = 1.209\ SL + 0.435$ | $TL = 1.207\ SL + 0.971$ |
| $r^2 = 0.986\ n = 852$ | $r^2 = 0.983\ n = 681$ | $r^2 = 0.985\ n = 1{,}533$ |
| $UCL = 3.693\ SL + 13.094$ | $UCL = 3.795\ SL + 10.452$ | $UCL = 3.758\ SL + 11.640$ |
| $r^2 = 0.874\ n = 507$ | $r^2 = 0.898\ n = 477$ | $r^2 = 0.903\ n = 1{,}054$ |
| $TL = 1.106\ FL + 0.052$ | $TL = 1.111\ FL - 0.870$ | $TL = 1.108\ FL - 0.480$ |
| $r^2 = 0.985\ n = 853$ | $r^2 = 0.984\ n = 911$ | $r^2 = 0.984\ n = 1{,}746$ |
| *Prionace glauca* | | |
| $FL = 1.076\ SL + 1.862$ | $FL = 1.080\ SL + 1.552$ | $FL = 1.079\ SL + 1.668$ |
| $r^2 = 0.993\ n = 1{,}043$ | $r^2 = 0.997\ n = 1{,}276$ | $r^2 = 0.996\ n = 2{,}319$ |
| $TL = 1.249\ SL + 7.476$ | $TL = 1.272\ SL + 4.466$ | $TL = 1.262\ SL + 5.746$ |
| $r^2 = 0.986\ n = 1{,}043$ | $r^2 = 0.993\ n = 1{,}272$ | $r^2 = 0.990\ n = 2{,}315$ |
| $UCL = 0.219\ SL + 4.861$ | $UCL = 0.316\ SL + 2.191$ | $UCL = 0.306\ SL + 3.288$ |
| $r^2 = 0.903\ n = 1{,}038$ | $r^2 = 0.948\ n = 1{,}264$ | $r^2 = 0.929\ n = 2{,}302$ |
| $TL = 1.158\ FL + 5.678$ | $TL = 1.117\ FL + 2.958$ | $TL = 1.167\ FL + 4.133$ |
| $r^2 = 0.988\ n = 1{,}043$ | $r^2 = 0.992\ n = 1{,}272$ | $r^2 = 0.990\ n = 2{,}315$ |
| *Alopias superciliosus* | | |
| $FL = 1.075\ SL + 3.346$ | $FL = 1.081\ SL + 3.324$ | $FL = 1.073\ SL + 4.150$ |
| $r^2 = 0.987\ n = 90$ | $r^2 = 0.980\ n = 76$ | $r^2 = 0.986\ n = 166$ |
| $TL = 1.939\ SL - 11.990$ | $TL = 1.897\ SL - 7.359$ | $TL = 1.937\ SL - 12.630$ |
| $r^2 = 0.976\ n = 77$ | $r^2 = 0.946\ n = 70$ | $r^2 = 0.970\ n = 147$ |
| $UCL = 0.967\ SL - 9.588$ | $UCL = 0.922\ SL - 5.353$ | $UCL = 0.966\ SL - 10.908$ |
| $r^2 = 0.952\ n = 75$ | $r^2 = 0.869\ n = 71$ | $r^2 = 0.932\ n = 146$ |
| $TL = 1.775\ FL - 13.007$ | $TL = 1.722\ FL - 7.295$ | $TL = 1.773\ FL - 14.456$ |
| $r^2 = 0.956\ n = 77$ | $r^2 = 0.923\ n = 70$ | $r^2 = 0.949\ n = 147$ |

| *Alopias vulpinus* | | | *Sphyrna zygaena* | | |
|---|---|---|---|---|---|
| Relation | $r^2$ | $n$ | Relation | $r^2$ | $n$ |
| $FL = 1.118\ SL - 2.29$ | 0.989 | 22 | $FL = 0.845\ SL + 1.077$ | 0.998 | 56 |
| $TL = 1.930\ SL + 9.331$ | 0.956 | 22 | $TL = 1.322\ SL + 8.397$ | 0.994 | 56 |
| $UCL = 0.926\ SL + 25.670$ | 0.949 | 22 | $UCL = 0.329\ SL + 8.799$ | 0.975 | 55 |
| $TL = 1.687\ FL + 20.483$ | 0.932 | 22 | $TL = 1.225\ FL + 7.528$ | 0.994 | 56 |

length for males (95 cm FL) was slightly greater than for females (80 cm FL), whereas in gillnet landings it was equivalent (100 cm FL). Mean lengths in the longline landings, including nonsexed specimens, ranged from 85 cm FL in sector 5 to 205 cm FL in sector 1. The mean length for gill nets was the same for

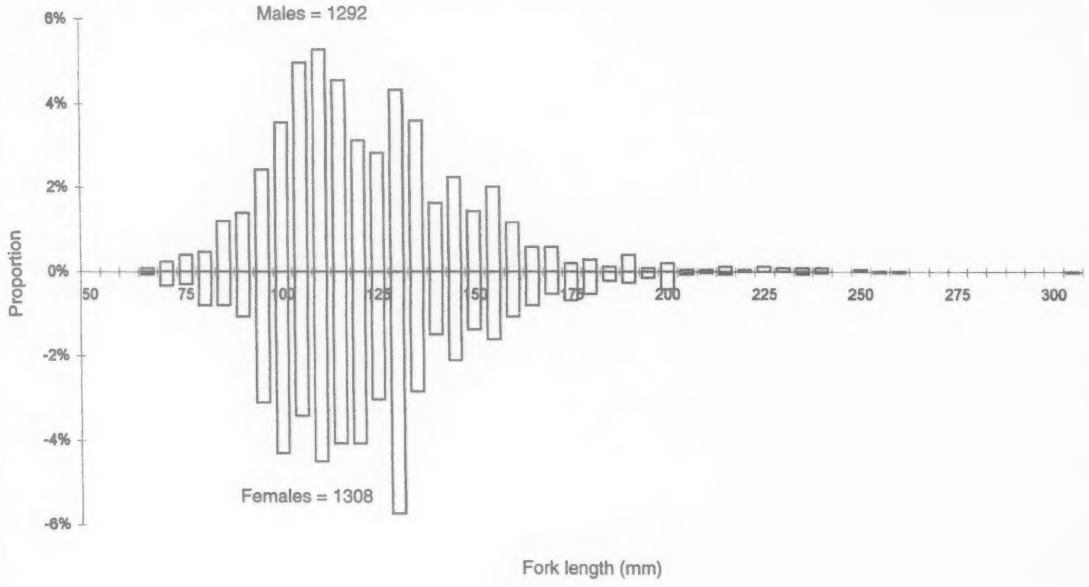


**Figure 3**

Total length-frequency distribution by sex of the shortfin mako shark longline landings from all sectors from July 1991 to July 1992.

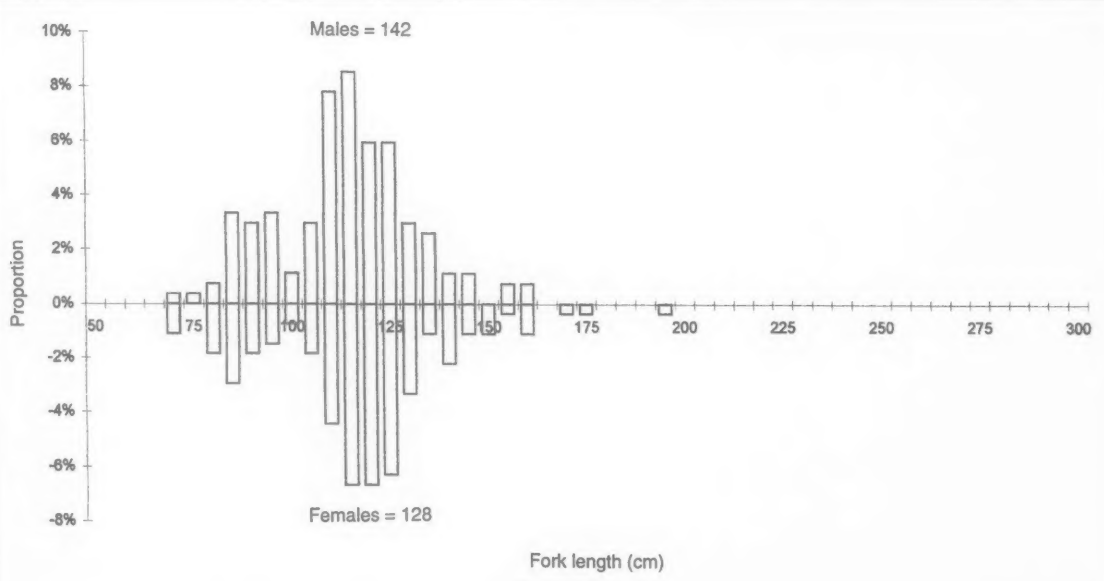


**Figure 4**

Total length-frequency distribution by sex of the shortfin mako shark gillnet landings from sector 5 from July 1991 to July 1992.

males and females (100 cm FL). The incompleteness of these data did not allow any temporal analyses.

Size distribution by sector varied significantly ($F$=387.130; df=5, 8214; $P$<0.001). Total mean size was greatest in sector 1, lowest in sector 5. Sector 3 had minimum-size females and males. The largest size females were found in sector 1; the largest males, in sector 4 (Fig. 7, B and C).

Table 8 shows biometrical comparisons by sex.

## Bigeye thresher shark

Longline catch rates for bigeye thresher sharks were very low. However, this species was caught in all sectors, sector 2 having the highest rate (Table 5). There was a slight increase in the longline catch rate, from

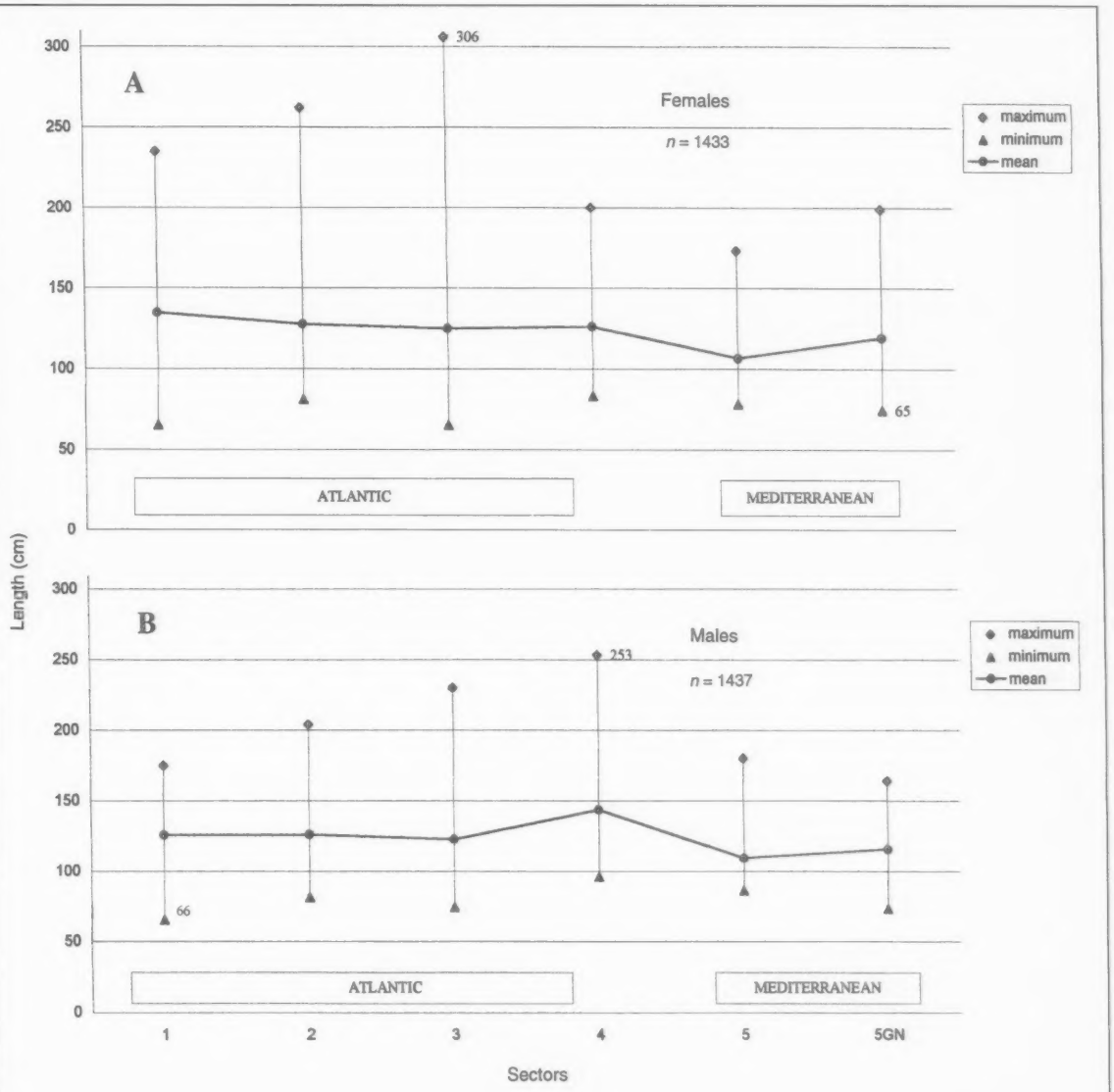


**Figure 5**

(**A**) Size range variation of shortfin mako shark females landed by longlines and gill nets (5GN) by sector from July 1991 to July 1992; (**B**) size range variation of the shortfin mako shark males landed by longlines and gill nets (5GN) by sector from July 1991 to July 1992.

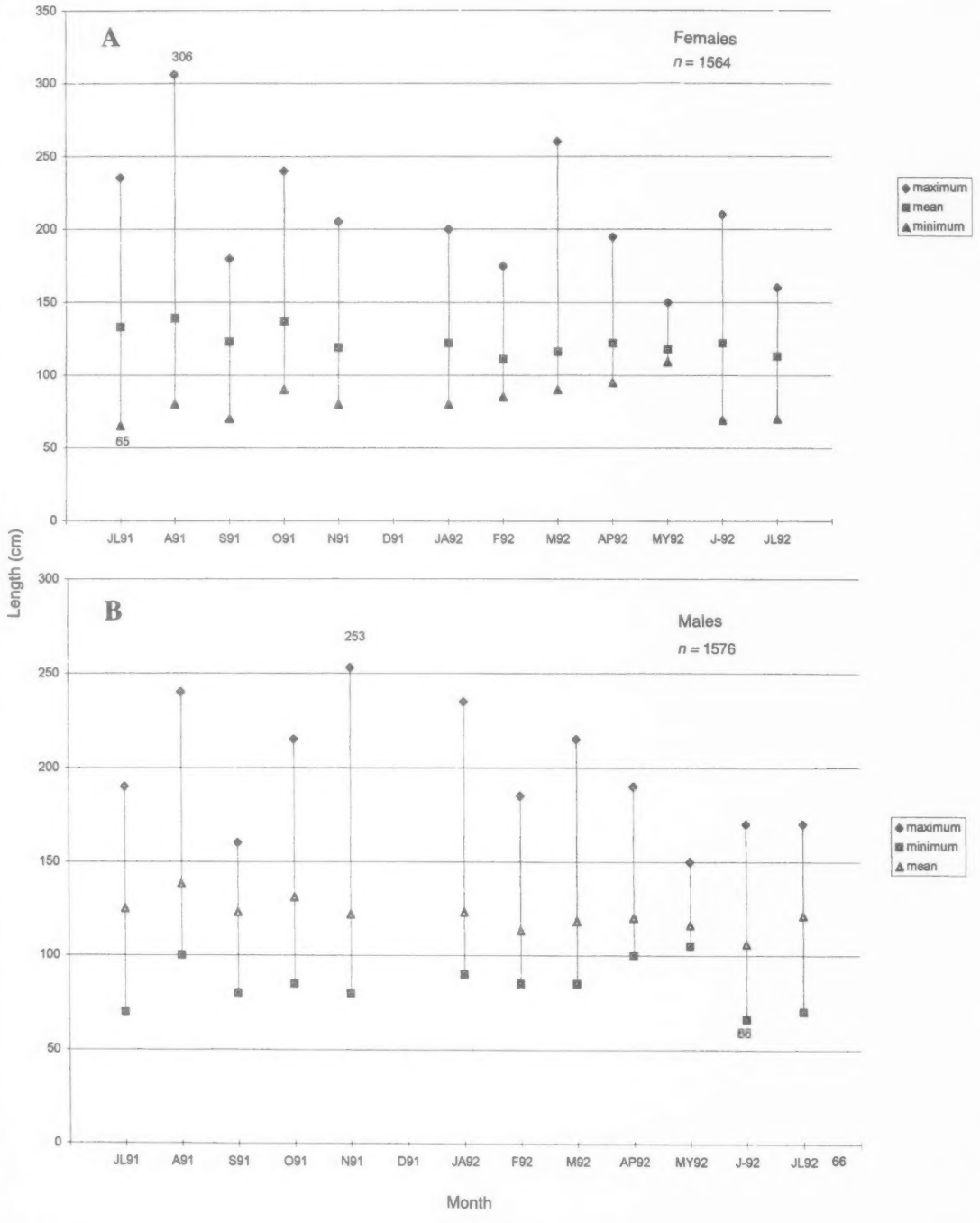


**Figure 6**

(**A**) Size range variation of the shortfin mako shark females landed by month (December 91 excluded); (**B**) size range variation of the shortfin mako shark males landed by month (December 91 excluded).

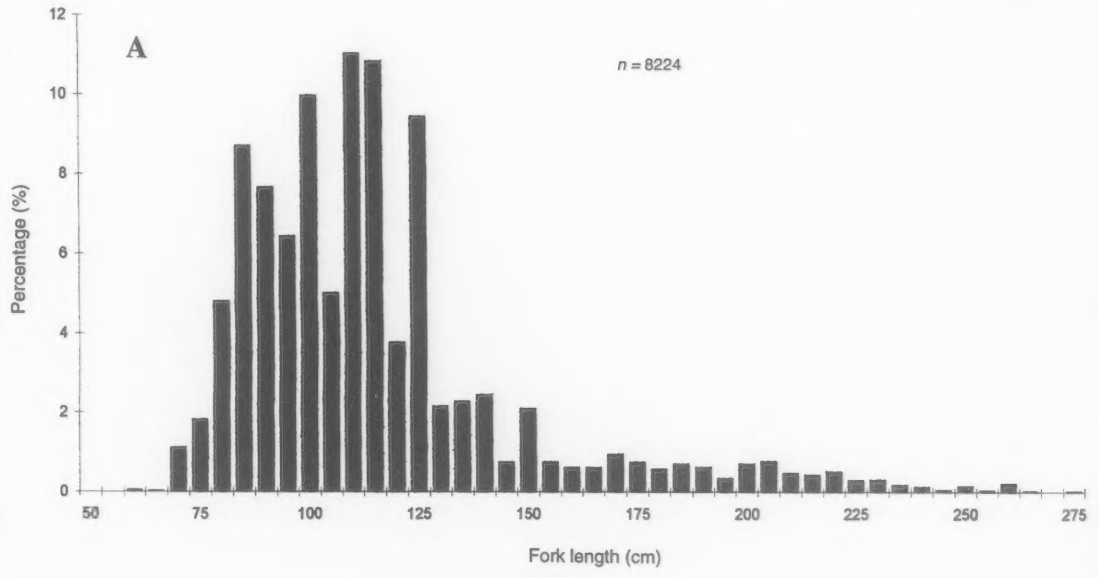


**Figure 7**

(**A**) Total length-frequency distribution of blue shark from longline and gillnet landings from all sectors from July 1991 to July 1992. (**B**) Size range variation of blue shark females landed by longlines and gill net (5GN) by sector from July 1991 to July 1992. (**C**) Size range variation of blue shark males landed by longlines and gill net (5GN) by sector from July 1991 to July 1992.

fall to winter, dropping during spring. A maximun catch rate was reached in July 1991 (Table 6). In gillnet fishing, catch rates were highest during summer months, with a maximum in July 1992 (Table 7).

The sex ratio found in longline fishing was fairly balanced, 1 male to 1.1 females in sector 2 to 1 to 0.93 females in sector 3. Females were more abundant than males in gillnet fishing (1 male:1.43 females). The distribution of sex ratio by sector was not significantly different ($\chi^2$=9.14; df=5; $P$>0.1). The distribution of sex ratio varied significantly by month ($\chi^2$=38.7; df=11; $P$<0.001). In longline fishing there were more males than females (1 male:0.66–0.69 females), except in February and March when the ratio was 1 male:1.45 females, and in September, when the proportion of females rose dramatically to 1 male:6.1 females. Gillnet fishing reflected a similar proportion during the same month (1 male:13.21 females).

The frequency distribution by size of fish from longline landings was quite similar for males and females (Fig. 8). Modal length for all sectors and months was near to 285 cm TL, the mean size approximately 280 cm TL. Minimum size was 180 cm TL for females, 195 cm TL for males. Maximum size was 40 cm greater for females (430 cm TL) than for males (390 cm TL).

The frequency distribution by size of fish from gillnet landings differed by sex (Fig. 9), with two modes for males (290 and 330 cm TL) and for females (395 and 410 cm TL).

The size range of fish by sector for longline landings was greater for females (183–432 cm TL) than for males (195–391 cm TL). Minimum sizes were found in sector 2 for both sexes, whereas the maximum for males was recorded in sector 3 and for females in sector 5. In gillnet landings, females ranged from 236 to 446 cm TL, with a mean size of 352 cm TL, 45 cm larger than the mean size of males (307 cm TL). As in longline landings, males had a smaller size range (246–373 cm TL). The length mode for females was 80 cm larger than for males (410 cm TL compared with 330 cm TL).

The size variation over time was not outstanding for males in either fishery, but there was a marked presence of large females during July–September in gillnet landings (288–446 cm TL) corroborated by longline landings in the same period (230–432 cm TL). The period of January to March was when the greatest number of fish were recorded (128) with the smallest size range (183–360 cm TL). Only one pregnant female (385 cm TL), with one pup, was recorded in gillnet landings in September 1991.

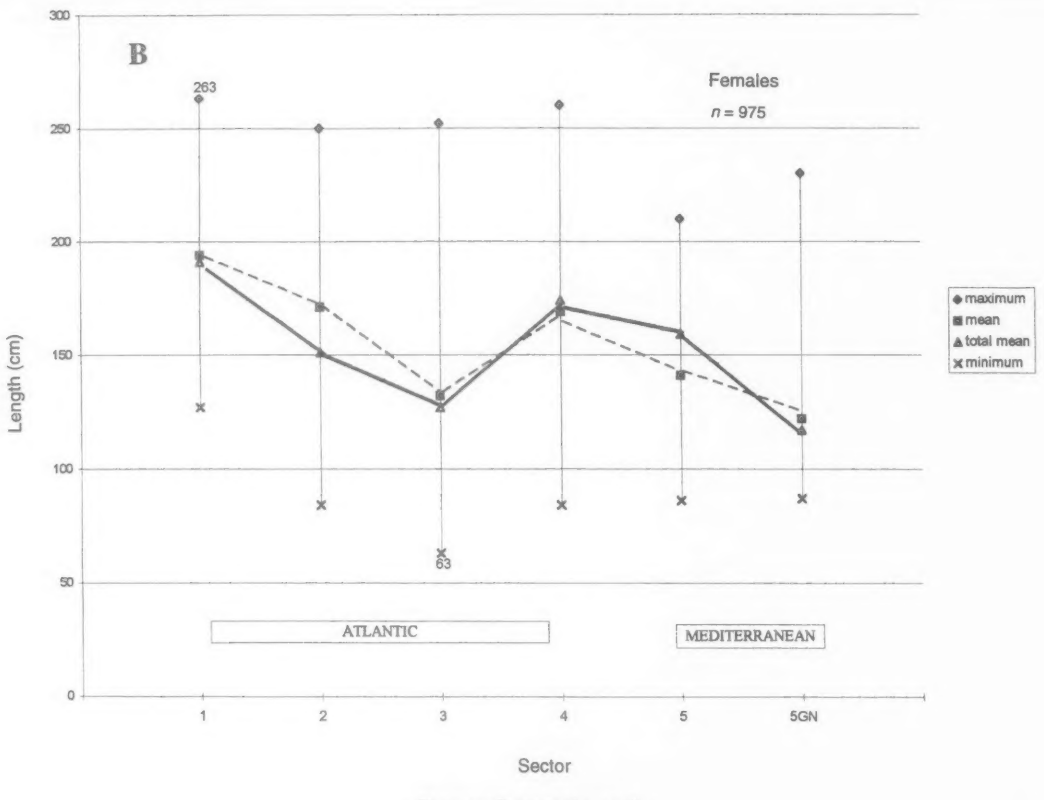


Figure 7 (continued)

## Common thresher shark

Only 52 common thresher sharks were recorded during the sampling period; hence the catch rate never went beyond 0.1 fish/1000 hooks (Tables 5 and 6). The overall sex ratio was close to 1 male:2 females, with some differences between longline (1 male:2.16 females) and gillnet (1 male:1.75 females) landings.

The size range of fish was similar for longline and gillnet landings, as was the mean size for both sexes (325 cm TL for males, 350 cm TL for females). Maximum size was similar in longline (360 cm TL for males, 425 cm TL for females) and gillnet landings (355 cm TL for males, 435 cm TL for females). Minimum size was comparable for females in both fisheries (295 and 285 cm TL in longline and gillnet, respectively), but not for males; in longline landings, a 245-cm-TL male was recorded, whereas in gillnet landings the minimum-size male recorded was about the same size as the minimum-size female (280 cm TL). Although biometrical information on this species is found in Table 8, our small sample size did not permit analyses by sex. Only one pregnant female was recorded, measuring 385 cm TL; it was caught in sector 4 by longline in May and carried 4 embryos (3 males and 1 female), 75 to 80 cm FL.

## Scalloped hammerhead shark

The catch rate for this species was low in all sectors and months, slightly greater for sectors 1 and 3 (0.6 and 0.8 fish/1000 hooks respectively), and never higher than 1 fish/1000 hooks (Tables 5–7). The highest overall catch rates by month in longline landings was in November 1991 and July 1992 (1.3 and 1.1 fish/1000 hooks). In gillnet landings the greatest catch rates were in September–October and June–July (0.1 fish/unit effort). Males were more abundant in the longline (1 male:0.61 females) than in the gillnet fishery, where females were dominant (1 male:1.37 females).

Females had a larger maximum size than males in both fisheries, 320 cm and 305 cm TL for females,

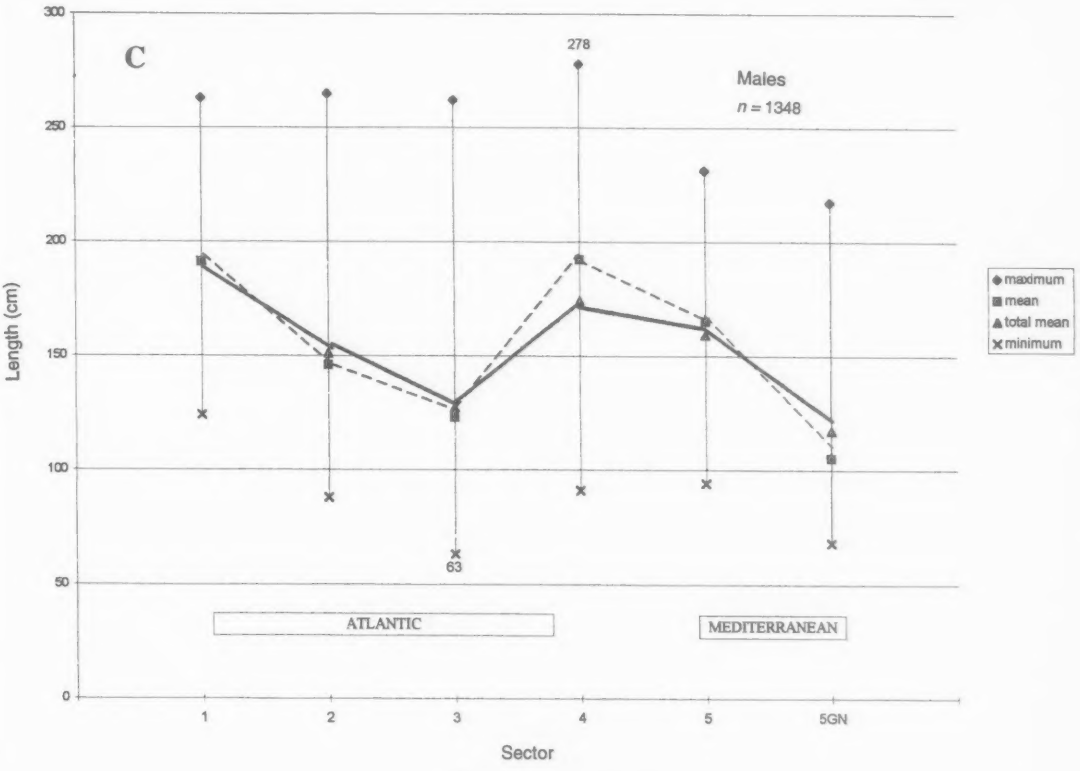


**Figure 7 (continued)**

280 and 275 cm TL for males, respectively, in longline and gillnet landings. Minimum size was greater in the gillnet fishery (185 cm TL for males, 165 cm TL for females) than in the longline fishery (105 cm TL for males, 115 cm TL for females). The mean size from longline landings was slightly greater for females (170 cm TL) than for males (150 cm TL). The mean size from the gillnet fishery was greater than from the longline fishery, with no difference between sexes (220 cm TL). Although biometrical information can be found in Table 8, the small sample size did not permit analyses by sex.

## Discussion

The total proportion of sharks in relation to swordfish observed in the present study was similar to that found by Gouveia (1992) for an adjacent area, but the species composition was different (*P. glauca*, *I. oxyrinchus*, *Dasyatis violacea*, *Alopias* spp., *Mustelus mustelus*, *Sphyrna* spp., *T. obesus*, and *Alepisaurus ferox*). Sharks comprised a greater proportion of landings in the study area than in other Atlantic longline fishing grounds, i.e. off the Florida coast (Berkeley and Campos, 1988), the Caribbean coast (Tobias, 1991), and the northwestern coast of Cuba (Guitart, 1975). "Other bony fish" (the other category of fish) taken by longlines had a similar proportion to that found in data from other Mediterranean longline fisheries (Rey and Alot, 1984). The abundance of the most common shark species, blue and shortfin mako sharks, also agrees with results presented for the same area by Garcés and Rey (1984).

### Shortfin mako shark

This species was very common in the study area. This finding agrees with reports by Muñoz-Chápuli (1985), although catch rates obtained were slightly lower than estimates of Moreno and Morón (1992b) from the same area. Proportions of shortfin mako sharks,

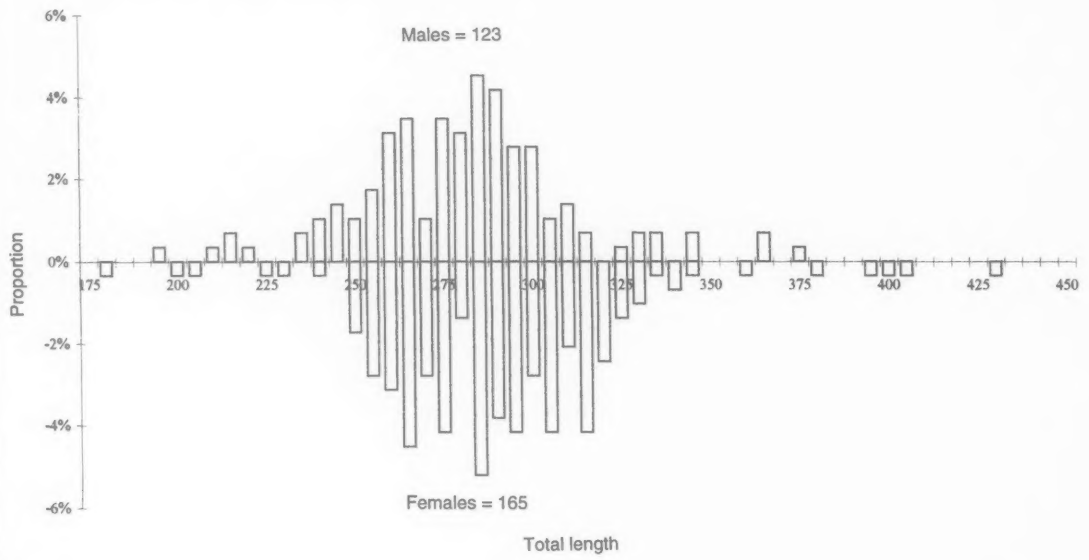


**Figure 8**

Total length-frequency distribution of the bigeye thresher shark by sex from longline landings from all sectors from July 1991 to July 1992.

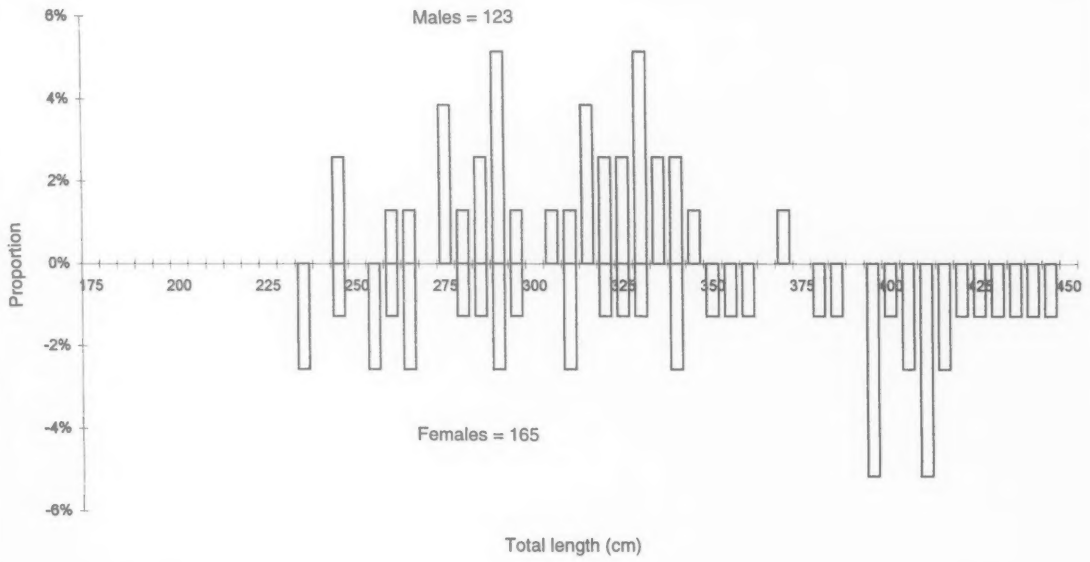


**Figure 9**

Total length frequency distribution of the bigeye thresher shark by sex from the gillnet landings from all the sectors from July 1991 to July 1992.

compared with swordfish, were similar to proportions calculated by Garcés and Rey (1983) for the northeastern Atlantic and Mediterranean Sea but were greater than the estimates of Rey and Alot (1984) for the western Mediterranean Sea and those of Mejuto[2] for the northeastern Atlantic just immediately north of the study area. Monthly variation in the longline catch rate in this study was different from that found off the northwestern Spanish coast by Mejuto and Garcés[1] and Mejuto[2], although it coincides with the increased trend observed during the last few months of the year. Monthly catch rate variation did not agree with results obtained on the other side of the Atlantic Ocean off the northwestern Cuban coast (Guitart, 1975). Shortfin mako shark and swordfish catch rates followed a similar decreasing trend from fall to March; in April the catch rate for shortfin mako sharks increased and that for swordfish dropped (Fig. 10), thus complementing each other.

Sex ratio (1 male:0.9 females) is different from that observed by Mejuto and Garcés[1] and Mejuto[2] in the northeastern Atlantic above 40°N, where males were more abundant (approx. 1 male:0.4 females). The increasing presence of males northwards (up to 1 male:0.6 females in sector 4) correlates with results observed by Mejuto and Garcés[1] and Mejuto[2] and may suggest sexual segregation to the north. For the same area of the present study, Muñoz-Chápuli (1984) observed a lower proportion of females (1 male:0.35 females) than we did, and although the sample size was small in that study ($n$=113), there was a similar trend in the increasing number of males northwards. The overall sex ratio observed in this study agrees with results presented by Moreno and Morón (1992b) from August 1983 to August 1985 for the same fleet operating in the same area. Mejuto[2] reported a trend of increase in percentage of males with increasing size (for size range 105–260 cm FL). This trend was not found in our study (Fig. 11).

The length-frequency distribution of the two fisheries was different from that found by Mejuto and Garcés[1] and Mejuto[2] for the area immediately north. It was also different from the one presented for the east coast of the United States (Pratt and Casey, 1983). In our study, modal values were always lower than those presented by the above-mentioned authors. Following the age structure proposed by Pratt and Casey (1983), we estimated that most of the fish in this study belonged to age class 1 and 2 for both sexes. The largest size of females corresponded to age class 5 (sector 3), and the largest size of males to

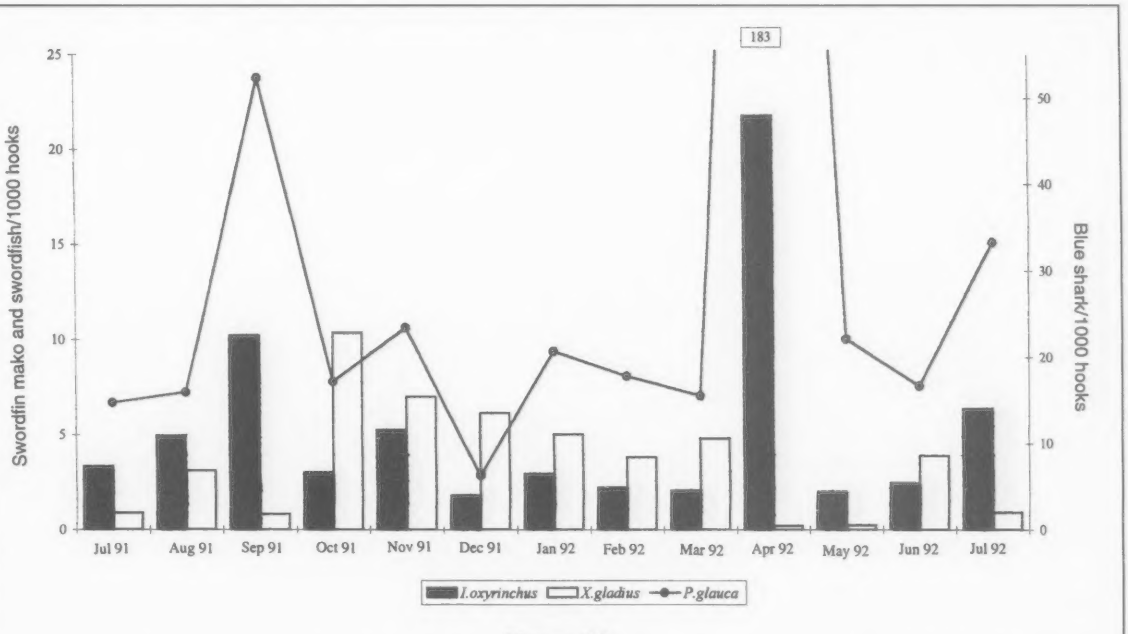


**Figure 10**

Comparison of longline catch rates (number of fish/1000 hooks) of the shortfin mako shark and swordfish (left "y" axis) and of the blue shark (right "y" axis) by month.

age class 4 (sector 4). The minimum size corresponded to age class 0 in all sectors.

Maximum size decreased during March–May (see Fig. 6, A and B). In June and July there was a possible entry of newborn fish (65 cm) with the same birth size as that suggested by Compagno (1984). Mean size decreased during October–November. These changes in size distribution suggest a movement of the largest and mean-size shortfin mako sharks out of the study area. For an area just north of the one studied, Mejuto[2] showed a large increase in catch rate during the last quarter of the year, which could be attributed partially to entry of fish coming from our study area.

### Blue shark

The abundance of this species in longline fishing has been mentioned by several authors (Bigelow and Schroeder, 1948; De Metrio et al., 1984; Muñoz-Chápuli, 1985; Stevens, 1990; Mejuto[2]). In the present study the proportion of blue sharks, compared with swordfish, was half that estimated by Garcés and Rey (1983) for an area that included our five sectors. However, it was greater than the proportion obtained by Rey and Alot (1984) in the western Mediterranean and very similar to that calculated by Mejuto[2] for the area just north of our study region. The number of fish landed increased northward and was greater than the number estimated by De Metrio et al. (1984) in the Mediterranean.

The temporal distribution of fish was also different for the Mediterranean (De Metrio et al., 1984) and for the northeastern Atlantic (Mejuto[2]). As in the case of shortfin mako sharks, the highest catch rates correlated with reductions in swordfish catch rates, i.e. April and September (Fig. 10).

The overall sex ratio (1 male:0.71 females) was inverse to that found by Rey and Alot (1984) in the Mediterranean. The latitudinal distribution of sexes was inverse to that estimated by Stevens (1990) off southwest Great Britain and Portugal and to that presented by Nakano et al. (1985) in a similar latitude in the central north Pacific.

Using Pratt (1979) and Cailliet et al's. (1983) age estimate, we found that frequency distribution of fish by size of landings in sector 1 showed that mature males were more abundant, whereas in the other sectors a greater proportion of immature fish were observed.

### Bigeye thresher shark

The relatively frequent occurrence of this species noted by Muñoz-Chápuli (1985) contrasted with the low numbers observed in our study, with the low

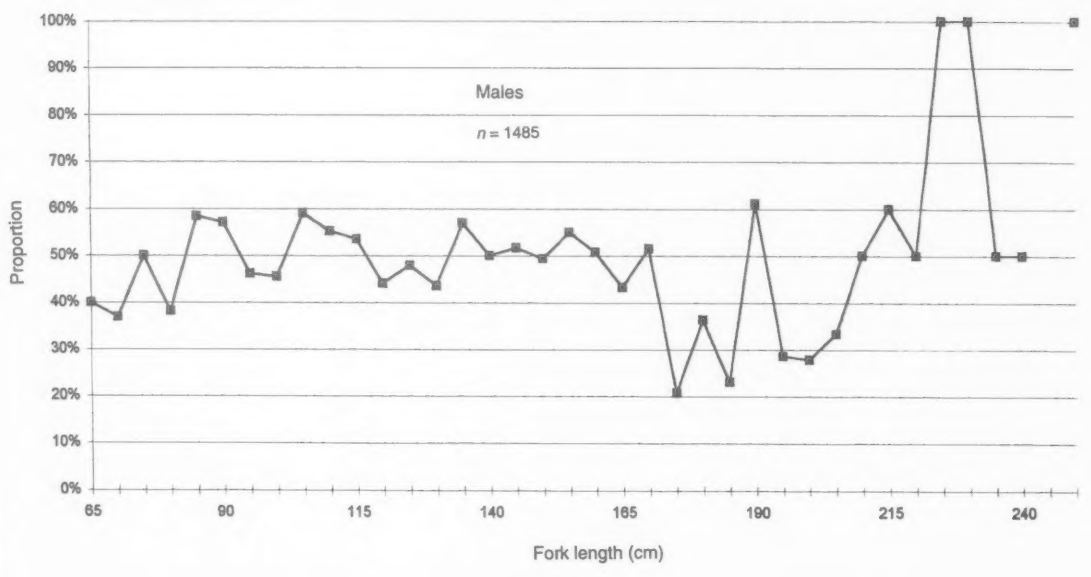


**Figure 11**

Proportion of male shortfin mako sharks in landings by size group (grouped by 5 cm FL).

numbers noted by Moreno and Morón (1992a) in the same area, and with the low numbers noted by Stillwell and Casey (1976) in the northwestern Atlantic. The sexual segregation hypothesized by Muñoz-Chápuli (1984) in this area, i.e. males distributed northward and females southward, was opposite that of our findings. Moreno and Morón (1992a) mentioned that most fish caught during the fall were pregnant females, whereas in this study mostly mature males from the longline fishery and mostly females from the gillnet fishery were recorded during the same period.

Most males in all sectors were adults (>276 cm TL, Moreno and Morón, 1992a). Most females would be have been considered mature in every sector according to Gubanov's (1979) first maturity size (>310 cm TL), but only in sector 5 according to Moreno and Morón's (1992a) criteria (340 cm TL). The largest fish was recorded in sector 5, a 432-cm-TL female, in September 1991, which, nevertheless, did not exceed the maximum for the species (460 cm TL, Nakamura, 1935).

Size distributions by month showed a greater proportion of immature males during the July–September period, whereas mature fish predominated the rest of the year. During January–June and October–December most females were around maturity size limit (300 cm TL, Gubanov, 1979).

Most births occur during fall and winter according to Moreno and Morón (1992a), and as these authors have suggested, sector 5 could be a breeding area. A pregnant female was recorded in September 1991, in sector 5, carrying only one pup. However, no newborn fish were recorded, according to birth sizes reported by Bigelow and Schroeder (1948), Bass et al. (1975), Gruber and Compagno (1981), and Moreno and Morón (1992a).

### Common thresher shark

The scarcity of this species in the present study disagrees with observations of Muñoz-Chápuli (1985). The relative abundance of common thresher sharks found in sector 5 was similar to that presented by Moreno et al. (1989) for the same area. The maximum number during spring agreed with the number observed in California (Cailliet and Bedford, 1983) but did not coincide with the peak reported by Moreno et al. (1989) during fall in this area.

The sex ratio for this species in the area studied (1 male:2 females) differed greatly from estimates by Holts (1988) on the west coast of the United States (1 male:1 female). Moreno et al. (1989) noted the absence of males in May, which suggests sexual segregation during the reproduction period. A mature male was recorded in May at 330 cm TL, which represents the lower limit of maturity for males according to Cailliet et al. (1983).

Only one female was recorded below size of first maturity (260 cm TL, established by Gubanov, 1972; Cailliet and Bedford, 1983; Cailliet et al., 1983). Moreno et al. (1989) pointed out the probable existence of a breeding area close to the Strait of Gibraltar during spring time. The record of a 425-cm-TL pregnant female, with four full-term pups in May, supports this theory.

### Scalloped hammerhead shark

According to sex and length data obtained from sectors 1, 3, and 5, the overall sex ratio (1 male:0.83 females) differed from the ratio observed by Muñoz-Chápuli (1984) in the same area (1 male:6 females).

If we follow Compagno's (1984) first maturity size criteria (256 cm TL for males, 304 cm TL for females), only 6% of males and 4% of females landed would be considered mature in our study area. Muñoz-Chápuli (1984) observed many pregnant females in the area throughout the year, but the lowest size recorded in our study (114 cm TL) was much greater than birth size (50–60 cm TL, Compagno, 1984). Moreover, the lack of mature females contradicts the assessment that the study area is a breeding area, as suggested by Muñoz-Chápuli (1985).

## Conclusions

Sharks, because of their low reproduction rate and late sexual maturity, are extremely sensitive to fishing pressure (Holden, 1973, 1974, 1977). The vulnerability of shark populations as bycatch of some tuna and tunalike fishing is comparable to that of marine mammals (Burke and Francis, 1990). In recent years several cases of overexploitation in shark fisheries (Cailliet and Bedford, 1983; Holts, 1988; Vas, 1990; Hanan et al., 1993) and the effect of other fishing activities (recreational fisheries, the routine procedure of discarding sharks after removal of fins) on shark populations (Casey and Hoey, 1985; Stevens, 1992) has been described.

A lack of fishery statistics about sharks is commonplace all around the world. Du Buit (1989) commented on the complete absence of knowledge about most biological factors influencing shark populations off the coast of France and on the difficulty in sampling most of these migratory species.

Our study shows the importance of shark landings in the Atlantic swordfish longline fishery and points out the large number of immature fish involved.

Shortfin mako may be the shark species most affected by current fishing procedure despite the fact that the most common species caught is blue shark. As Casey and Kohler (1992) suggested with reference to the shortfin mako shark population in western north Atlantic, this species has a continuous distribution throughout the central and northeastern Atlantic Ocean, preferentially in the high production area of Saharian Bank (sector 3).

As it has been shown in this study, sharks greatly affect and are greatly affected by swordfish fishing. Therefore, the management of this fishing industry should be reoriented to multispecies models in which the effect of bycatch and the economic implications of bycatch should be included in the management models for a proper approach to the present situation in the industry.

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## Literature cited

**Amorín, A., C. Arfelli, A. Garcés, and J. C. Rey.**
**1979.** Estudio comparativo sobre la biología y pesca del pez espada, *Xiphias gladius* L. (1758) obtenidos por las flotas españolas y brasileña. Col. Vol. Sci. Pap. ICCAT 8(2):496–503.

**Anonymous.**
**1986.** Anuario de pesca Marítima 1985. Secretaría General Técnica. Ministerio de Agricultura, Pesca y Alimentación, Madrid, 501 p.

**Bass, A. J., J. D. D'Aubrey, and N. Kistnasamy.**
**1975.** Sharks of the east coast of southern Africa. IV. The families Odontaspididae, Scapanorhynchidae, Isuridae, Cetorhinidae, Alopiidae, Orectolobidae and Rhiniodontidae. Inv. Rep. Oceanogr. Res. Inst., Durban 39:1–102.

**Belloc, G.**
**1934.** Catalogue illustré des poissons comestibles de la côte occidentale d'Afrique (du Cap Spartel au Cap Vert). Première partie, Poissons cartilagineux. Rep. Trav. Off. (Scient. Tech.) Pêch. Marit. 7(2):117–195.

**Berkeley, S. A., and W. L. Campos.**
**1988.** Relative abundance and fishery potential of pelagic sharks along Florida's East Coast. Mar. Fish. Rev. 50(1):9–16.

**Bigelow, H., and W. C. Schroeder.**
**1948.** Fishes of the western north Atlantic. Pt. 1: sharks. Mem. Sears Found. Mar. Res. I:59–576.

**Blasco, M., and R. Muñoz-Chápuli.**
**1981.** Presencia de *Alopias superciliosus* en las costas ibéricas y datos sobre su morfología. Arq. Mus. Bocage, ser. B. 1(6):53–61.

**Bravo, J.**
**1974.** Contribución al conocimiento de los Peces Condroíctios del Archipiélago Canario. Bol. Inst. Espa. Oceanogr. 201:1–77.

**Bravo, J., and E. Santaella.**
**1973.** Observaciones biológico pesqueras en el banco pesquero sahariano. Bol. Inst. Espa. Oceanogr. 171:1–79.

**Burke, T. W., and T. C. Francis.**
**1990.** Options for the management of tuna fisheries in the Indian Ocean. FAO Fish. Tech. Pap. 135:1–74.

**Cailliet, G. M., and D. W. Bedford.**
**1983.** The biology of three pelagic sharks from California waters, and their emerging fisheries: a review. CalcCOFI Rep. 24:57–69.

**Cailliet, G. M., L. K. Martin, J. T. Harvey, D. Kusher, and B. A. Welden.**
**1983.** Preliminary studies on the age and growth of blue, *Prionace glauca*, common thresher, *Alopias vulpinus*, and shortfin mako, *Isurus oxyrinchus*, sharks from California waters. *In* E. Prince and L. M. Pulos, (eds.), Proceedings of the international workshop on age determination of oceanic pelagic fishes: tunas, billfishes, and sharks, p. 179–188. U.S. Dep. Commer. NOAA Tech. Rep. NMFS 8.

**Casey, J. G., and J. J. Hoey.**
**1985.** Estimated catches of large sharks by U.S. recreational fishermen in the Atlantic and Gulf of Mexico. U.S. Dep. Commerce, NOAA Tech. Rep. 31:15–19.

**Casey, J. G., and N. E. Kohler.**
**1992.** Tagging studies on the shortfin mako shark (*Isurus oxyrinchus*) in the western north Atlantic. Aust. J. Mar. Freshwater Res. 43:45–60.

**Compagno, L. J. V.**
**1984.** FAO species catalogue. Vol. 4: Sharks of the world: an annotated and illustrated catalogue of sharks species known to date. Part 2: Carcharhiniformes. FAO Fish. Synop. 125, p. 251–655.

**De Metrio, G., G. Petrosino, C. Montanaro, A. Matarrese, M. Lenti, and E. Cecere.**
**1984.** Survey on summer–autumn population of *Prionace glauca* L. Pisces, Chondrichthyes) in the Gulf of Taranto (Italy) during the four year period 1978–1981 and its incidence on sword fish (*Xiphias gladius* L.) and albacore (*Thunnus alalunga* (Bonn)) fishing. Oebalia 10 N.S.:105–116.

**Du Buit, M. H.**
**1989.** L'exploitation des Selaciens en France. Océanis 15(3):333–344.

**Garcés, A. G., and J. C. Rey.**
**1983.** Análisis de la pesquería española de pez espada, *Xiphias gladius*, entre los años 1973 y 1981. Col. Vol. Sci. Pap. ICCAT 18:622–628.
**1984.** La pesquería española del pez espada (*Xiphias gladius*) 1973–1982. Col. Vol. Sci. Pap. ICCAT 20:419–427.

**García, C.**
**1970.** La pesca en Canarias y en el banco sahariano. Publ. Cons. Eco. Soc. Sind. Inter. Canarias. Tenerife, 168 p.

**Gouveia, L.**
**1992.** Swordfish (*Xiphias gladius*, Linnaeus) fishing experiment in Madeira EEZ. Col. Vol. Sci. Pap. ICCAT 39(2):477–483.
**Gruber, S. H., and L. J. V. Compagno.**
**1981.** Taxonomic status and biology of the bigeye thresher, *Alopias superciliosus*. Fish Bull. 79(4):617–639.
**Gubanov, Y. P.**
**1972.** On the biology of the thresher shark *Alopias vulpinus* in the northwest Indian Ocean. J. Ichthyol. 12:75.
**1979.** The reproduction of some species of pelagic sharks from the equatorial zone of the Indian Ocean. J. Ichthyol. 18:781–792.
**Guitart, D.**
**1975.** Las pesquerías pelágico-oceánicas de corto radio de acción en la región noroccidental de Cuba. Acad. Cien. Cuba Ser. Oceanol. 31:1–26.
**Hanan, D. A., D. B. Holts, and A. L. Coan.**
**1993.** The California drift gill net fishery for sharks and swordfish, 1981–82 through 1990–91. Fish Bull. 175: 1–95.
**Holden, M. J.**
**1973.** Are long-term sustainable fisheries for elasmobranchs possible? Rapp. P.-V Cons. Int. Explor. Mer 164:360–367.
**1974.** Problems in the rational exploitation of elasmobranch populations and some suggested solutions. *In* F. R. Harden-Jones (ed.), Sea Fisheries Research, p. 117–137. Paul Elek, London.
**1977.** Elasmobranchs. *In* J. A. Gulland (ed.), Fish population dynamics, p. 187–215. John Wiley and Sons, New York, NY.
**Holts, D. B.**
**1988.** Review of U. S. west coast commercial shark fisheries. Mar. Fish. Rev. 50(1):1–8.
**Lozano Cabo, F.**
**1950.** Datos sobre la repartición geográfica de especies de peces de la costa de NW Africa. Bol. R. Soc. Espa. Hist. Nat. Sec. Biol. 48(1):5–14.
**Moreno, J. A.**
**1982.** Jaquetones. tiburones del género *Carcharhinus* del Atlántico Nor-Oriental y Mediterráneo Occidental. Ministerio de Agricultura, Pesca y Alimentación, Madrid, 205 p.
**Moreno, J. A., and J. Morón.**
**1992a.** Reproductive biology of the begeye thresher shark, *Alopias superciliosus* (Lowe, 1839). Aust. J. Freshwater Res. 43:77–86.
**1992b.** Comparative study of the genus *Isurus* (Rafinesque, 1810), and description of a form ("Marrajo Criollo") apparently endemic to the Azores. Aust. J. Mar. Freshwater Res. 43:109–122.
**Moreno, J. A., J. I. Parajuá, and J. Morón.**
**1989.** Biología reproductiva y fenología de *Alopias vulpinus* (Bonnaterre,1788) (Squaliformes: Alopiidae) en el Atlántico nor-oriental y Mediterráneo occidental. Scient. Mar. 53(1):37–46.
**Muñoz-Chápuli, R.**
**1984.** Ethologie de la reproduction chez quelques requins de l'Atlantique Nord-Est Cybium 8(3):1–14.
**1985.** Análisis de las capturas de escualos pelágicos en el Atlántico nororiental (15°–40°N). Inv. Pesq. 49(1):67–79.
**Muñoz-Chápuli, R., and M. Blasco.**
**1984.** Tendencias generales del crecimiento relativo en escualos. Inv. Pesq. 48(3):303–317.
**Nakamura, H.**
**1935.** On the two species of the thresher shark from Formosan waters. Mem. Fac. Sci. Agric. Taihoku Imp. Univ. 14(1):1–6.
**Nakano, H., M. Makihara, and K. Shimaziki.**
**1985.** Distribution and biological characteristics of the blue shark in the central north Pacific. Bull. Fac. Fish. Hokkaido Univ. 36(3):99–113.
**Pratt, H. L.**
**1979.** Reproduction in the blue shark, *Prionace glauca*. Fish. Bull. 77:445–470.
**Pratt, H. L., and J. G. Casey.**
**1983.** Age and growth of the shortfin mako, *Isurus oxyrinchus*, using four methods. Can. J. Fish. Aquat. Sci. 40(11):1944–1957.
**Rey, J. C., and E. Alot.**
**1984.** Contribución al estudio de la pesquería de palangre del pez espada (*Xiphias gladius*) en el Mediterráneo Occidental. Col. Vol. Sci. Pap. ICCAT 20:428–434.
**Stevens, J. D.**
**1990.** Further results from a tagging study of pelagic sharks in the north-East Atlantic. J. Mar. Biol. Assoc. (U.K.) 70:707–720.
**1992.** Blue and mako shark bycatch in the Japanese longline fishery off south-eastern Australia. Aust. J. Mar. Freshwater. Res. 43:227–236.
**Stillwell, C. E., and J. G. Casey.**
**1976.** Observations on the bigeye thresher shark, *Alopias superciliosus*, in the western north Atlantic. Fish. Bull. 74(1):221–225.
**Tobias, W.**
**1991.** Billfish bycatch observer data of the U.S. swordfish longline fleet, St. Croix, U.S. Virgin Islands—1988 and 1989. Col. Vol. Sci Pap. ICCAT 35(2):518–523.
**Vas, P.**
**1990.** The abundance of the blue shark, *Prionace glauca*, in the western English Channel. Env. Biol. Fishes 29: 209–225.

# Monophyly and intrarelationships of the family Pleuronectidae (Pleuronectiformes), with a revised classification

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**Abstract.**—A cladistic analysis of interrelationships for 53 (of 59) pleuronectid species was performed by using 106 morphological and osteological characters. The analysis resulted in 128 equally parsimonious cladograms (heuristic search, 403 steps, consistency index=0.33, retention index=0.79). A 50% majority-rule consensus cladogram indicated that only five of 47 resolved nodes were observed in less than 100% of the cladograms. These five nodes are restricted to interrelationships within one subfamily. The Pleuronectidae is monophyletic according to ten synapomorphies. In addition, five subfamilies were defined: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae. The largest subfamily, the Pleuronectinae, was further subdivided into four tribes: Psettichthyini, Isopsettini, Microstomini, and Pleuronectini. The interrelationships established within Pleuronectidae provide a strong foundation for a simplified yet phylogenetically informative taxonomic nomenclature at the genus-group level. The following genera are reclassified: *Atherestes* and *Reinhardtius* to *Reinhardtius*; *Errex*, *Glyptocephalus*, and *Tanakius* to *Glyptocephalus*; *Embassichthys*, and *Microstomus* to *Microstomus*; *Hypsopsetta* and *Pleuronichthys* to *Pleuronichthys*; and *Kareius* and *Platichthy* to *Platichthys*. To preserve the monophyletic status of *Eopsetta*, *E. exilis* was reassigned to the genus *Lyopsetta* (Lyopsettinae). The genus *Pleuronectes* (as defined by Sakamoto in 1984) was found to be polyphyletic. Monophyly of this genus is established by revising it to include only five species; *Pleuronectes glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, and *P. quadrituberculatus*. Other species, formerly placed in *Pleuronectes*, are now reclassified to *Isopsetta*, *Limanda*, *Parophrys*, *Psettichthys*, and *Pseudopleuronectes*. The monophyletic status of *Limanda* (six species) is uncertain because of unresolved relationships between these species and other taxa in the tribe Pleuronectini.

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The Pleuronectidae (*sensu* Chapleau and Keast, 1988) contains 59 nominal species of right-eyed flatfishes distributed in marine waters of the Northern Hemisphere. As presently composed, this family excludes the subfamilies Poecilopsettinae, Samarinae, Rhombosoleinae, and Paralichthodinae, formerly included in Norman (1934). It contains many commercial species that have long been harvested in coastal seas off Europe, North America, and Asia. Species such as the Petrale sole (*Eopsetta jordani*), Pacific halibut (*Hippoglossus stenolepis*), American plaice (*Hippoglossoides platessoides*), and Dover sole (*Microstomus pacificus*), to name but a few, are valued for their large size and excellent meat (Hart, 1973; Scott and Scott, 1988). In total, there are over 36 species of flatfish monitored by the Food and Agricultural Organization of the United Nations, with a total annual catch of 256,353 metric tons (t) in 1995 (FAO, 1997). Nearly one half (18) of these species are classified within the Pleuronectidae (FAO, 1997). Total annual commerical harvest of Pacific halibut (*Hippoglossus stenolepis*) was estimated at 25,968 t in 1995 a decrease from 40,584 t in 1985 (FAO, 1997). A similar situation has been observed in witch flounder (*Glyptocephalus cynoglossus*) for which 19,537 t was harvested in 1995 as compared to 30,074 t in 1985 (FAO, 1997). With few exceptions, this decline in annual harvest is observed for most pleuronectid species (FAO, 1997). The commercial popularity of pleuronectid species, coupled with a need to manage these renewable resources, strongly emphasizes the necessity for a better understanding of relationships within this group, as well as an informative taxonomic nomenclature that will provide a framework for management policy. Variation in life history traits are observed at many phylogenetic levels. Populations of the American plaice (*Hippoglossoides platessoides*) are recorded to have age of maturity ranging from 3 to 15 years (Roff, 1981). Within the family, variation in maximum length ranges from 220 mm in *Dexistes rikuzenius* (Sakamoto, 1984b) to over 2500 mm in *Hippoglossus stenolepis* (Hart, 1973). An hypothesis of species interrelationships can be used as a framework to assess phylogenetic constraint on life history traits versus a species' ability to respond to changing environmental conditions (Brooks and McLennan, 1991). An assessment of

phylogenetic constraint versus environmental influence could provide a more informed understanding of observed changes in life history traits.

The objectives of this study are to clarify the monophyletic status of the Pleuronectidae, to offer an hypothesis of relationships within the group based on adult morphology, and to establish a phylogenetically informative nomenclature. To attain these objectives, a reassessment of morphological evidence in the literature and new morphological characters were compiled in a matrix analyzed within a cladistic framework. A new classification based on the phylogenetic information is offered.

## Historical classification and diagnosis of Pleuronectidae

The Pleuronectidae was regarded by early ichthyologists to represent all known flatfishes (Norman, 1934). For example, Cuvier (1816) subdivided the Pleuronectidae into five subfamilies: Hippoglossinae, Pleuronectinae, Platessinae, Soleinae, and Cynoglossinae; as did Jordan and Goss (1889) who also added the subfamilies: Samarinae and Oncopterinae. Changes to the early classification in flatfish focused on revisions that accommodated new species without a complete revision of the entire scheme. Newly discovered species, thought to represent distinct morphological groups, were classified into new subgroups; but original species, and those that did not have special morphologies, were to remain within the Pleuronectidae. Thus, the Pleuronectidae became a "garbage" group.

Jordan and Evermann (1898) raised flatfishes to the suborder Heterosomata with two distinct families: Pleuronectidae and Soleidae. The Pleuronectidae, with three subfamilies: Hippoglossinae, Pleuronectinae, and Psettinae, were characterized by "a more or less distinct preopercular margin (i.e. not hidden by the skin and scales of the head); eyes large, well separated; mouth moderate or large; teeth present" (Jordan and Evermann, 1898). The Soleidae were subdivided into two subfamilies, Soleinae and Cynoglossinae, and were characterized by "an adnate preopercular margin, hidden by the skin and scales of the head; eyes small, situated close together; mouth very small, much twisted; teeth rudimentary or wanting" (Jordan and Evermann, 1898).

Regan (1910) proposed a new classification that raised the Heterosomata to the level of order with two suborders: Psettodoidea and Pleuronectoidea. Within the second suborder, the Pleuronectidae now contained three subfamilies; Pleuronectinae, Samarinae, and Rhombosoleinae. The family was characterized by "having eyes on right side of head, nerve of left eye always dorsal, olfactory lamellae slightly raised, parallel without central rachis and eggs without oil globules" (Regan, 1910).

This classification was adopted by Norman (1934), who incorporated minor revisions from Regan (1920, 1929) and Jordan (1923). The Pleuronectidae, at this point containing five subfamilies (Pleuronectinae, Samarinae, Rhombosoleinae, Poecilopsettinae, and Paralichthodinae) were characterized by Norman (1934) as "having eyes on the right side; optic chiasma monomorphic, the nerve of the left eye always dorsal; dorsal fin extending forward on the head at least to above the eye; all the fin-rays articulated; pelvic of from 3 to 13 rays; mouth usually terminal, with the lower jaw more or less prominent; maxillary without a supplemental bone; palatines toothless; lower edge of urohyal deeply emarginate, so that the bone appears forked; preoperculum with free margin; nasal organ of blind side usually near edge of head, but sometimes nearly opposite that of ocular side; vertebrae never fewer than 30; on each side a single post-cleithrum; ribs present; egg without an oil-globule in the yolk." Later classifications removed the genera *Brachypleura* and *Lepidoblepharon* from the Pleuronectidae and placed them in the Citharidae (Hubbs, 1945) but essentially agreed with the classification proposed by Norman (1934).

Nelson (1984) listed the Poecilopsettinae, Rhombosoleinae, Samarinae, and Pleuronectinae as subfamilies in Pleuronectidae on the basis of two characteristics: eyes almost always dextral and no oil globule in yolk of egg. Sakamoto's (1984a) hypothesis of pleuronectid intrarelationships assumed that the Pleuronectinae, Samarinae, Rhombosoleinae, Poecilopsettinae, and Paralichthodinae were monophyletic because both eyes were on right side of the body, optic nerve of the left eye was always dorsal, preopercle had a free margin and fin rays were without spines. Hensley and Ahlstrom (1984), in a review of flatfish classification, indicated that the evidence for monophyly of Pleuronectidae (*sensu* Norman, 1934) was not convincing. The diagnostic characters reviewed in Norman (1934) were found to be plesiomorphic for the order or had distributions that were unknown for many pleuronectiform taxa (Hensley and Ahlstrom, 1984).

Subsequent cladistic analysis of major taxa within the order supported the hypothesis that the Pleuronectidae was not monophyletic and suggested that the subfamilies Pleuronectinae, Samarinae, Rhombosoleinae, and Poecilopsettinae should be elevated to the family level (Chapleau and Keast, 1988; Chapleau, 1993). This new interpretation of taxonomic ranks in right-eyed flounders was recognized

by Hensley (1993) and in part by Nelson (1994). Species in Samarinae of Nelson (1984) and *Paralichthodes algoensis* were classified to Samaridae (Nelson, 1994). The subfamilies Pleuronectinae, Rhombosoleinae, and Poecilopsettinae, remained in Pleuronectidae. Nelson (1994) argued that without a comprehensive understanding of monophyly for some major groups it is difficult to provide an accurate revision of the nomenclature within the order. Although this approach is an obvious attempt to minimize unnecessary changes to the nomenclature, it does not reflect the understanding that only species in Pleuronectinae possess a bothoid caudal-fin complex that clearly distinguishes these 59 north temperate species as being closely related to Bothidae, Paralichthyidae, Scophthalmidae, and *Brachypleura* (Hensley and Ahlstrom, 1984; Chapleau, 1993). The Poecilopsettinae, Rhombosoleinae, and Samaridae do not have this caudal-fin complex and are phylogenetically related to Achiridae, Soleidae, and Cynoglossidae (Chapleau, 1993). Given that Pleuronectinae is the nominotypical subgroup, it is correct to reclassify it to Pleuronectidae. For the species of Poecilopsettinae, and Rhombosoleinae, it is a misrepresentation of the present cladistic framework to classify them in Pleuronectidae when the order level phylogeny (Chapleau, 1993) suggests a relationship with Samaridae, Achiridae, Soleidae, and Cynoglossidae. Therefore, only the 59 nominal species of Pleuronectinae are considered in this study and classified as Pleuronectidae (*sensu* Chapleau and Keast, 1988).

Two tribes in Pleuronectinae (*sensu* Norman, 1934), the Hippoglossini and Pleuronectini, were classified on the basis of jaw morphological characters (Nelson, 1984). The Hippoglossini identified by "mouth large and symmetrical; maxillae extending to or behind pupil of eyes; teeth well developed on both sides of jaws, contained ten genera (e.g. *Atherestes*, *Eopsetta*, *Hippoglossoides*, *Hippoglossus*, *Lyopsetta*, *Psettichthys*, and *Reinhardtius*)." The Pleuronectini were identified by "mouth small and asymmetrical; maxillae usually not extending to pupil of eye; teeth chiefly on blind-side of jaw and contained 16 genera (e.g. *Embassichthys*, *Glyptocephalus*, *Hypsopsetta*, *Isopsetta*, *Lepidopsetta*, *Limanda*, *Liopsetta*, *Microstomus*, *Parophrys*, *Platichthys*, *Pleuronectes*, *Pleuronichthys*, and *Pseudopleuronectes*)" (Nelson, 1984). Although this classification was effective in identifying two morphological types within Pleuronectinae (*sensu* Norman, 1934), it was not based on an examination of interrelationships within the group, nor did it accurately identify natural groups. The characters defining Hippoglossini were all plesiomorphic for the order and the characters defining the Pleuronectini were also observed in many lineages closely related to the Pleuronectinae (*sensu* Norman, 1934).

The 59 nominal species in this group had been historically classified in as many as 28 genera, many of which were monotypic. This nomenclature was established prior to any understanding of phylogeny and reflected the morphological diversity within Pleuronectidae. The number of genera used in identifying pleuronectid species would presumably be used to accommodate new species as they were discovered. However, the alpha taxonomy for this group has been well established, and only one new species of Pleuronectidae, *Microstomus shuntovi* Borets, 1983, has been described in the latter half of this century. Intuitively, a simplified and more informative nomenclature with fewer monotypic genera would seem appropriate.

There are few published studies that have dealt with phylogenetic relationships among pleuronectid taxa. The most extensive examination of interrelationships and classification within Pleuronectidae (*sensu* Norman, 1934) was established by Sakamoto (1984a). This phenetic hypothesis of interrelationships among 77 species was not aimed at defining relationships within an evolutionary framework; nor was it aimed at determining taxonomic structure on the basis of natural groups. Sakamoto (1984a) concluded his study with a reclassification of several genera within the Pleuronectinae (*sensu* Norman, 1934). In revision, the species of *Eopsetta* and *Lyopsetta*, as well as *Cleisthenes* and *Hippoglossoides*, were reclassified into *Eopsetta* and *Hippoglossoides*. *Glyptocephalus zachirus* became *Errex zachirus*. All species of *Isopsetta*, *Parophrys*, *Lepidopsetta*, *Limanda*, *Pseudopleuronectes*, *Pleuronectes*, and *Liopsetta* were regrouped under the genus *Pleuronectes*. Finally *Paralichthodes algoensis*, previously classified in its own subfamily, Paralichthodinae (Nelson, 1994), was placed within the Pleuronectinae on the basis of overall similarity and the presence of the first neural arch, a symplesiomorphy for the order (Hensley and Ahlstrom, 1984).

Chiu (1990) examined the relationships among four glyptocephaline species (formerly classified in *Glyptocephalus* and *Tanakius*). The results of this phenetic analysis of body shape were similar to Sakamoto's (1984a) results with respect to the relationships between *Glyptocephalus cynoglossus*, *G. stelleri*, and *G. zachirus*. The limited scope of this analysis does not provide adequate information to infer relationships beyond these three species.

Sakamoto's (1984a) nomenclatural changes and classification were adopted in the American Fisheries Society checklist for flatfish species (Robins et

al., 1991). The adoption of this reclassification was a recognition of the first and only study that attempted to define intrarelationships in the Pleuronectidae (*sensu* Norman, 1934). This new classification has not been widely accepted (Wheeler, 1992; Rass, 1996). It is argued here that because the Pleuronectidae used by Sakamoto (1984a) was determined to be polyphyletic (Chapleau, 1993) and given the phenetic nature of the analysis, it is unlikely that the nomenclatorial revisions summarized in that work represent natural groups.

The dubious nature of this most recent reclassification (i.e. Sakamoto, 1984a), the uninformative nature of the previous classification (i.e. Norman, 1934), and the commercial importance of this group require that a more comprehensive examination of pleuronectid intrarelationships be based on natural groups. A cladistic analysis based on structural variation within Pleuronectidae, in contrast with characters observed in closely related outgroups will establish an hypothesis of genealogical descent (Wiley, 1981).

## Outgroup hypothesis for Pleuronectidae

Relationships within the order reveal that only the Pleuronectidae (*sensu* Chapleau and Keast, 1988) have a caudal skeletal complex, synapomorphic with taxa belonging to the Paralichthyidae, Scophthalmidae, *Brachypleura*, and Bothidae (Chapleau, 1993). These taxa have been identified as the bothoid lineage within Pleuronectiformes (Hensley and Ahlstrom, 1984). This lineage is supported in one of 18 equally parsimonious trees observed in a cladistic analysis for the order (Chapleau, 1993). The other taxa formerly in Pleuronectidae (Samaridae, Rhombosoleidae, and Poecilopsettidae) were placed in a clade that included the soles Achiridae, Soleidae, and Cynoglossidae (Chapleau, 1993). *Paralichthodes algoensis* was not included in Chapleau's (1993) cladistic revision but has recently been determined to be the sister lineage to these other taxa formerly in Pleuronectidae (Cooper and Chapleau, 1998). Consequently, the most likely outgroup for Pleuronectidae would be represented by species within the bothoid lineage.

There is a wide range of morphological types within the bothoid lineage for which the intrarelationships are not resolved (Chapleau, 1993). In the absence of synapomorphies to determine the sister relationship of Pleuronectidae with other bothoid taxa, a comparison of jaw structure can be used to determine the most likely candidates. It is assumed that the outgroup for the Pleuronectidae should have large symmetrical jaws and pointed teeth. Within Pleuronectiformes, the evolutionary trend for jaw structure and feeding strategy may be considered unidirectional. Symmetry of jaw and dentition found in piscivorous flatfishes, like Psettodidae and Citharidae (de Groot, 1971), is considered to be the plesiomorphic condition. The osteological characters observed in these two taxa are most similar to the generalized acanthopterygian structure (Yazdani, 1969). Taxa with symmetrical jaw structure are hypothesized to have given rise to groups with more specialized dentition types and jaw asymmetry, as observed in the Achiridae, Soleidae, and Cynoglossidae (Yazdani, 1969; Chapleau, 1993), but the reverse situation is not indicated in any study of relationships.

Within subgroups of Pleuronectiformes, the same evolutionary trend is assumed to occur. Left-eyed flounders within the bothoid lineage have large, nearly symmetrical jaws for piscivory, or a more specialized, asymmetrical jaw structure that accommodates capture of benthic prey (Yazdani, 1969; de Groot, 1971). Likewise, the Pleuronectidae contains both piscivores with nearly symmetrical jaws, *Hippoglossus stenolepis* and *Reinhardtius hippoglossoides,* as well as more specialized predators with asymmetrical jaws, such as *Glyptocephalus stelleri* (de Groot, 1971). Assuming that evolutionary trends in pleuronectid jaw structure are consistent with trends observed in the order, the ancestral pleuronectid would have near symmetrically developed jaws. The bothoid family, Paralichthyidae, appears to be one of the most plesiomorphic groups of left-eyed flounders and is chosen as the outgroup for the Pleuronectidae. In addition, *Psettodes* and *Lepidoblepharon* are also chosen as secondary outgroups. These assumptions are only valid if the Pleuronectidae is monophyletic. If Pleuronectidae is not monophyletic, then multiple outgroup taxa with either symmetrical or asymmetrical jaw structures may account for the variation observed within the Pleuronectidae.

## Materials and methods

Fifty-three of 59 pleuronectid species were examined. Five outgroup taxa, chosen from the families Psettodidae (*Psettodes* sp.), Citharidae (*Lepidoblepharon ophthalmolepis*), and Paralichthyidae (*Citharichthys arenaceus*, *Paralichthys lethostigmus*, and *P. squamilentus*) were also examined. The following cleared and stained specimens were dissected and examined for osteological characters. Nomenclature follows the conclusions of this analysis with the previous classification of Sakamoto (1984a) indicated in

parentheses. Changes in species-group nomenclature recognize gender status of the genus (Eschmeyer, 1990) as specified by the International Code of Zoological Nomenclature, article 31 (Ride et al., 1985). Institutional abbreviations follow Leviton et al. (1985). Length, in mm, is standard length (Hubbs and Lagler, 1970). Radiographs for all specimens, as well as radiographs of the specimens listed in Leipertz (1987), were also examined.

**Psettodidae** *Psettodes* sp. Bennet; ANSP 145394, 65 mm. **Citharidae** *Lepidoblepharon ophthalmolepis* Weber; AMS I.20118-012, 122 mm. **Paralichthyidae** *Citharichthys arenaceus* Evermann and Marsh; USNM 00203510, 69 mm. *Paralichthys lethostigmus* (Jordan and Gilbert); ANSP 143209, 90 mm. *Paralichthys squamilentus* Jordan and Gilbert; ANSP 150694, 50 mm. **Pleuronectidae** *Acanthopsetta nadeshnyi* Schmidt; USNM 77122, 89 mm. UW 22792, 224 mm; *Cleisthenes* (=*Hippoglossoides*) *herzensteini* (Schmidt); USNM 051441, 85, 89 mm. *Cleisthenes* (=*Hippoglossoides*) *pinetorum* Jordan and Starks; UMMZ 159566, 76 mm. *Clidoderma asperrimum* (Temminck and Schlegel); not examined. *Dexistes rikuzenius* Jordan and Starks; UMMZ 159662, 122 mm. *Eopsetta grigorjewi* (Herzenstein); UMMZ 159590, 66, 88 mm. *Eopsetta jordani* (Lockington). NMC 81-1015, 68 mm. *Glyptocephalus cynoglossus* (Linnæus); NMC 77-1087, 89, 114 mm. *Glyptocephalus kitaharai* (Jordan and Starks); UMMZ 141741, 139 mm. *Glyptocephalus stelleri* (Schmidt); UMMZ 159566, 125 mm. *Glyptocephalus* (=*Errex*) *zachirus* Lockington; NMC 65-0211, 95 mm; NMC 81-1027, 133 mm. *Hippoglossoides dubius* Schmidt; not examined. *Hippoglossoides elassodon* Jordan and Gilbert; NMC 61-0117, 71, 82 mm. *Hippoglossoides platessoides* (Fabricius); NMC 80-0601, 31, 61, 63, 77 mm; ROM 504CS, 73 mm; ROM 786CS, 70, 83, 87, 90 mm. *Hippoglossoides robustus* Gill and Townsend; ANSP 105133, 110 mm. *Hippoglossus hippoglossus* (Linnæus); ARC 8808487, 148 mm. *Hippoglossus stenolepis* Schmidt; NMC 61-0072, 100 mm; UW 22743, 54, 56, 67 mm. *Isopsetta* (=*Pleuronectes*) *isolepis* (Lockington); UMMZ 63214, 119, 123 mm. *Lepidopsetta* (=*Pleuronectes*) *bilineata* (Ayres); NMC 61-0050, 51 mm; NMC 81-1027, 56 mm. *Lepidopsetta* (=*Pleuronectes*) *mochigarei* Snyder; UMMZ 159575, 113 mm. *Limanda* (=*Pleuronectes*) *aspera* (Pallas); NMC 66-0016, 156 mm. *Limanda* (=*Pleuronectes*) *ferruginea* (Storer); NMC 80-0217, 102, 107 mm; ROM 560CS, 49 mm. *Limanda* (=*Pleuronectes*) *limanda* (Linnæus); MNHN 1959-560, 119 mm; *Limanda* (=*Pleuronectes*) *proboscidea* Gilbert. USNM 268496, 141 mm; UW 22742, 98, 115 mm. *Limanda* (=*Pleuronectes*) *punctatissima* (Steindachner); HUMZ 93958, 135 mm. *Limanda* (=*Pleuronectes*) *sakhalinensis* Hubbs; HUMZ 60455, 138 mm. *Lyopsetta* (=*Eopsetta*) *exilis* (Jordan and Gilbert); NMC 60-0501, 111, 115 mm. *Microstomus achne* (Jordan and Starks); UMMZ 159434, 145 mm. *Microstomus* (=*Embassichthys*) *bathybius* (Gilbert); UW 22791, 167 mm. *Microstomus kitt* (Walbaum); FMNH 35527, 135 mm. *Microstomus pacificus* (Lockington). NMC 81-1027, 109 mm. *Microstomus shuntovi* Borets; not examined. *Parophrys* (=*Pleuronectes*) *vetula* Girard; FMNH 97128, 97, 128 mm; NMC 81-1121, 39, 61, 66, 68 mm; NMC 85-0025, 53, 67 mm. *Platichthys* (=*Kareius*) *bicoloratus* (Basilewsky); UMMZ 159667, 117 mm. *Platichthys flesus* (Linnæus); ANSP 93141, 78 mm. *Platichthys stellatus* (Pallas); NMC 61-0044, 53, 85, 90 mm. *Pleuronectes glacialis* Pallas; NMC 62-0352, 72 mm. *Pleuronectes pinnifasciatus* (Kner) Steindachner and Kner. HUMZ 75681, 150 mm. *Pleuronectes platessus* (Linnæus); ANSP 93145, 66, 88 mm. *Pleuronectes putnami* (Gill); ROM 23214, 28 mm, ROM 556CS, 104, 110 mm. *Pleuronectes quadrituberculatus* Bean; USNM 064042, 85 mm. *Pleuronichthys coenosus* Girard; not examined. *Pleuronichthys cornutus* (Temminck and Schlegel); UMMZ 159618, 91 mm. *Pleuronichthys decurrens* Jordan and Gilbert; CAS 23703, 58 mm. *Pleuronichthys* (=*Hypsopsetta*) *guttulatus* Girard; NMC 74-0242, 64 mm. *Pleuronichthys ocellatus* Starks and Thompson; CAS 82189, 105 mm. *Pleuronichthys ritteri* Starks and Morris; CAS 11403, 46 mm. *Pleuronichthys verticalis* Jordan and Gilbert; CAS 34728, 83 mm. *Psettichthys melanostictus* Girard; NMC 62-2158, 58, 63 mm. *Pseudopleuronectes* (= *Pleuronectes*) *americanus* (Walbaum); ANSP 105133, 43, 66 mm; NMC 82-0016, 61, 73 mm; ROM 670CS , 27, 35, 46, 49, 54, 60 mm. *Pseudopleuronectes* (=*Pleuronectes*) *herzensteini* (Jordan and Snyder); UMMZ 159631, 88 mm. *Pseudopleuronectes* (=*Pleuronectes*) *obscurus* (Herzenstein); not examined. *Pseudopleuronectes* (=*Pleuronectes*) *schrenki* (Schmidt); HUMZ 75697, 123 mm. *Pseudopleuronectes* (=*Pleuronectes*) *yokohamae* (Günther); UMMZ 159548, 58, 83 mm; UMMZ 220249, 74 mm; USNM 056359 86 mm. *Reinhardtius* (=*Atheresthes*) *evermanni* (Jordan and Starks); not examined. *Reinhardtius hippoglossoides* (Walbaum); NMC 64-0756, 103, 119 mm. *Reinhardtius* (=*Atheresthes*) *stomias* (Jordan and Gilbert); NMC 65-0262, 97 mm; NMC 66-0022, 39 mm; NMC 80-0073, 80, 117 mm; NMC 80-1024, 119 mm. *Verasper moseri* [Jordan and Gilbert] Jordan and Evermann; USNM 056385, 78 mm. *Verasper variegatus* (Temminck and Schlegel); USNM 056375, 91 mm.

All characters used in the study are described in the Appendix. This list also includes the number of steps and character consistency index (cci) for each character in the analysis. The character matrix

(Table 1), illustrating the distribution of 106 characters for 58 taxa, combines information from the literature (Norman, 1934; Batts, 1964; Amaoka, 1969; Ahlstrom et al., 1984; Hensley and Ahlstrom, 1984; Sakamoto, 1984a) as well as 67 new morphological features observed through examination of cleared and stained material, whole preserved specimens, and radiographs. Morphological states obtained from the literature are indicated by the citation immediately following the italic character description (Appendix). Morphological states based on meristic counts represented the modal value of the sample population. Characters at each node of the cladogram are described and numbered in order of presentation in the text.

Given the unresolved nature of the interrelationships for Pleuronectidae and other bothoid taxa within the order (Chapleau, 1993), the establishment of character polarity by outgroup comparison, as outlined by Watrous and Wheeler (1981) and Maddison et al. (1984), was not possible. Character polarity was determined through a direct examination of states observed in outgroup taxa (Table 1). For each character, the majority state observed in the three bothoid taxa *Citharichthys arenaceus*, *Paralichthys lethostigmus*, and *P. squamilentus* was assumed to represent the plesiomorphic condition. This decision was only overruled if there was heterogeneity in the distribution of states within these three taxa and if both secondary outgroup taxa *Lepidoblepharon ophthalmolepis* and *Psettodes* sp. possessed the alternative state. One exception to this rule is stated in character 82 (Appendix). Character states hypothesized as plesiomorphic for the family are coded as zero (0).

### Heuristic search methods

The matrix was analyzed with all combinations of heuristic search parameters available in PAUP 3.1.1.[1] An exhaustive search of the most parsimonious tree with this many taxa (53) would have required the analysis of an estimated $2.84 \times 10^{82}$ bifurcating trees (Felsenstein, 1978) and is not possible within the current standard of computational time. For these same reasons, a branch and bound search technique proved inadequate to resolve relationships for more than 25 taxa (Forey et al., 1992). A two-step procedure (a sequential addition of taxa to produce a cladogram that minimizes homoplasy followed by the subsequent branch-swapping of this addition tree to search for more parsimonious cladograms) was used to search for the most parsimonious result. The random addition sequence in combination with all of the branch-swapping algorithms was a nonrigorous means of assessing the efficiency of the heuristic methods (Forey et al., 1992). If 50 random replicates give the same set of tree topologies, then it is likely that the maximally parsimonious trees have been found. However, if after 100 replicates shorter cladograms are still being found, then it is likely that more trees remain (Forey et al., 1992).

To minimize confounding effects of local minima, the "keep" option was used on successive searches to allow swapping on nonminimal trees (Forey et. al., 1992). Both the "MULPARS" and "swap on all trees" options were employed during each heuristic search to minimize the effect of plateau (Forey et. al., 1992). All searches assumed that the ingroup was monophyletic, and all uninformative characters were ignored. Character optimization was set for accelerated transformation (ACCTRAN). The five outgroup taxa were not included in the analysis. Instead, ancestral states for all characters were set as zero according to established character polarity to represent a hypothetical outgroup. All most parsimonious trees were saved from each search and combined (without duplication) to establish a 50% majority-rule consensus of the equally parsimonious results. Character analysis, character consistency index (cci), tree statistics, and tree presentations were generated with MacClade version 3.04 (Maddison and Maddison, 1992).

## Results and discussion

### Phylogenetic analysis

The heuristic searches found multiple trees of equal length (Table 2). The most parsimonious trees were found to be 403 steps from a minimum of 131 steps, with a consistency index (ci) of 0.33, excluding uninformative characters, and a retention index (ri) of 0.79. Additional rounds of heuristic search allowed swapping on nonminimal trees up to 410 steps but did not resolve cladograms shorter than 403 steps. The "simple" and "closest" addition sequences were biased by taxa with large numbers of unknown character states. Criteria for establishing the initial tree and the addition of taxa are strongly influenced by unknown character states in these two algorithms. As a result, *Reinhardtius evermanni*, *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Microstomus shuntovi*, *Pleuronichthys coenosus*, and *Pseudopleuronectes obscurus* were not included in the heuristic searches. The "as is" and "simple" addition se-

[1] Swofford, D. L. 1991. PAUP Version 3.1.1. Unpublished software documentation.

**Table 1**

Matrix of 106 morphological characters for 5 outgroup taxa, representing the Psettodidae, Citharidae, and Paralichthyidae and 53 ingroup
Blanks represent the hypothesized ancestral state (state 0). Question marks (?) indicate an unknown state. Character states deemed not

| Character number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Psettodidae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Psettodes* sp. | | ? | | | 1 | | | 1 | | | 1 | | | na | | | | | 1 | | | 1 | | | 1 | | | | | | | na | na | na | | | | | | 1 | | | | | 1 | | | | |
| Citharidae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lepidoblepharon ophthalmolepis* | 1 | | | | | | | | | | | | | na | | 1 | | | | | | 1 | | | | | | | | | | na | na | na | | | | | | 1 | | | | 1 | | | 1 | | |
| Paralichthyidae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 1 | | | | | | 1 | 2 | | | | | | | 1 | | | |
| *Citharichthys arenaceus* | | | | | | | | | | | | 1 | 1 | 2 | | | 1 | | | 1 | 1 | | | | | | | | | 1 | 1 | | 1 | | | | | 1 | | | | | | | | | | |
| *Paralichthys lethostigmus* | | | | | | | 1 | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *P. squamilentus* | | | | | 1 | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pleuronectidae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 1 | 1 | | 1 | |
| *Acanthopsetta nadeshnyi* | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | 1 | | | | | | | | | | | 1 | | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | |
| *Cleisthenes herzensteini* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | | | | | | | 1 | | 1 | ? | ? | ? | 1 | 1 | 1 | 1 | |
| *C. pinetorum* | 1 | 1 | ? | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ? | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | ? | 1 | | | 1 | | | | | | | | ? | | | | 1 | | 1 | | | | | | | | |
| *Dexistes rikuzenius* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | 1 | | | 1 | | 1 | | | 1 | | | | 1 | 1 | 1 | |
| *Eopsetta grigorjewi* | 1 | 1 | 1 | | 1 | | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | |
| *E. jordani* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ? | 1 | | ? | | | | | | | ? | | | | | | | | ? | ? | ? | ? | | ? | | | ? | 1 | | ? | ? | | ? | 1 | | |
| *Glyptocephalus cynoglossus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 | 1 | | | 2 | | 1 | | 1 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | | | |
| *G. kitaharai* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | | 1 | 1 | | | 1 | 1 | | 1 | | | | 1 | 1 | 1 | | 1 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | | | |
| *G. stelleri* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 | 1 | | | 2 | | 1 | | 1 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | | | |
| *G. zachirus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | 1 | 2 | | | 1 | 1 | | 1 | 1 | | | 2 | | 1 | | 1 | 1 | | | 1 | | | 1 | 1 | | 1 | | | | 1 | | | |
| *Hippoglossoides elassodon* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | 1 | 1 | | 1 | 1 | |
| *H. platessoides* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | 1 | | 1 | 1 | |
| *H. robustus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | | | | | | | | | | | | | | 1 | | 1 | 1 | |
| *Hippoglossus hippoglossus* | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | | | | | 1 | | | | | | | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | 1 | | |
| *H. stenolepis* | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | | | | | 1 | | | | | | | 1 | 2 | 1 | | | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | 1 | | |
| *Isopsetta isolepis* | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | 1 | 1 | | | | | | 1 | | | | | | | 1 | | |
| *Lepidopsetta bilineata* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | ? | 1 | | | | | | | 1 | 1 | | | | | 1 | | | | | | | | ? | 1 | |
| *L. mochigarei* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | 1 | 1 | | | | | 1 | | | | | | | | 1 | 1 | |
| *Limanda aspera* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | | 1 | | | | | 1 | | | 1 | | 1 | 1 | 1 | | 1 | 1 | 2 |
| *L. ferruginea* | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | | | | | 1 | | | 1 | | 1 | | | | 1 | 1 | 2 |
| *L. limanda* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | | 1 | | | 1 | 1 | | | | | | | | 1 | 2 |
| *L. proboscidea* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | | | | | 1 | 1 | | | | | | | | 1 | 2 |
| *L. punctatissima* | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | | | | | | | | 1 | | | | | 1 | 1 | | | | | | | | 1 | 2 |
| *L. sakhalinensis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | | | | | 1 | 1 | | | | | | | | 1 | 2 |
| *Lyopsetta exillis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| *Microstomus achne* | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | | 1 | | | 2 | | | 1 | 1 | 2 | 1 | 1 | | | 1 | | 1 | | 1 | 1 | | | 1 | | | 1 | | | 1 | | | 1 | 1 | 1 | | |
| *M. bathybius* | 1 | 1 | 1 | 1 | 1 | 1 | | | | 1 | 1 | ? | | 1 | ? | | 2 | 1 | | 1 | 1 | 2 | 1 | 1 | | 2 | | 1 | | 1 | 1 | | | 1 | ? | | 1 | ? | | 1 | | | 1 | 1 | 1 | 1 | |
| *M. kitt* | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | | | 2 | | | 1 | 1 | 2 | 1 | 1 | | 1 | | 1 | | 1 | 1 | | | 1 | | | 1 | | 1 | | 1 | 1 | | 1 | | |
| *M. pacificus* | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | | | 2 | | | 1 | 1 | 2 | 1 | 1 | | 1 | | 1 | | 1 | 1 | | | 1 | | | 1 | | 1 | | 1 | 1 | 1 | 1 | | |
| *Parophrys vetula* | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | 1 | | | | | 1 | | | | | | | 1 | 1 | 2 |
| *Platichthys bicoloratus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | | | | | | | | | 2 | 2 | 1 | | 1 | | 1 | 1 | 1 | 2 | | 1 | ? | ? | | 1 | 1 | 3 |
| *P. flesus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | 1 | | | | 2 | 2 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 3 |
| *P. stellatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 2 | 2 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 3 |
| *Pleuronectes glacialis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 3 |
| *P. pinnifasciatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 3 |
| *P. platessus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | 1 | 2 | | | 1 | | 1 | 1 | 2 | | 1 | 1 | | | 1 | 1 | 3 |
| *P. putnami* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 3 |
| *P. quadituberculatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | ? | 1 | 2 | 2 | 1 | 1 | 1 | ? | 1 | 1 | | | | | | ? | ? | ? | ? | | 1 | | ? | 1 | 1 | 2 | ? | 1 | | ? | ? | | 1 | 1 | 2 |
| *Pleuronichthys cornutus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | | | | | 1 | | 1 | 1 | 1 | | 1 | | ? | 1 | 1 | 2 | ? | 1 | | ? | | | 1 | | |
| *P. decurrens* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | | 1 | 2 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | | | | | 1 | | 1 | 1 | 1 | | 1 | | 1 | 1 | 1 | | | | | | 1 | | |
| *P. guttulatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | | 1 | | | 1 | ? | 1 | 1 | | 1 | 1 | | | 1 | 1 | | | | | 1 | | 1 | 1 | | | | | 2 | 1 | | 1 | | | | | | | |
| *P. ocellatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | | | | | 1 | | 1 | 1 | | 1 | | | 1 | 1 | | | | | | | | 1 | | |
| *P. ritteri* | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | | | 1 | 2 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | | | | 1 | | 1 | 1 | 1 | 1 | | 1 | | 1 | 1 | | 1 | | | | | | 1 | | |
| *P. verticalis* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | | | | | 1 | | 1 | 1 | 1 | | 1 | | 1 | 1 | | 1 | | | | | | 1 | |
| *Psettichthys melanostictus* | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | | | | | 1 | | | 1 | 1 | 2 | | | | | 1 | 1 | 2 |
| *Pseudopleuronectes americanus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 2 |
| *P. herzensteini* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | | | | 1 | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | 1 | | | 1 | 1 | 2 |
| *P. schrenki* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 2 |
| *P. yokohamae* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | | | | | | | | 1 | 1 | | | 1 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | 2 |
| *Reinhardtius hippoglossoides* | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | 1 | ? | 2 | 1 | 1 | 2 | 1 | | | | | | | | | | | | | | 1 | | 1 | 1 | | 1 | |
| *R. stomias* | 1 | 1 | | | | 1 | | 1 | 1 | 1 | | | | | 1 | | | | | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | 1 | | | | | | | | | 1 | | | 1 | |
| *Verasper moseri* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | 1 | | | 1 | | | | | | 1 | 1 | | | | | 1 | 1 | 2 | 2 | 1 | | 1 | 1 | 1 | 1 | 2 | | 1 | | | | | 1 | |
| *V. variegatus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | 1 | | | | | | 1 | 1 | | | | | 1 | 1 | 2 | 2 | 1 | | 1 | 1 | 1 | 1 | 2 | | 1 | | | | | 1 | 1 |

taxa representing the Pleuronectidae. Numbers in the matrix represent character state for each morphology as described in the Appendix. applicable due to extreme variation in morphology are coded "na."

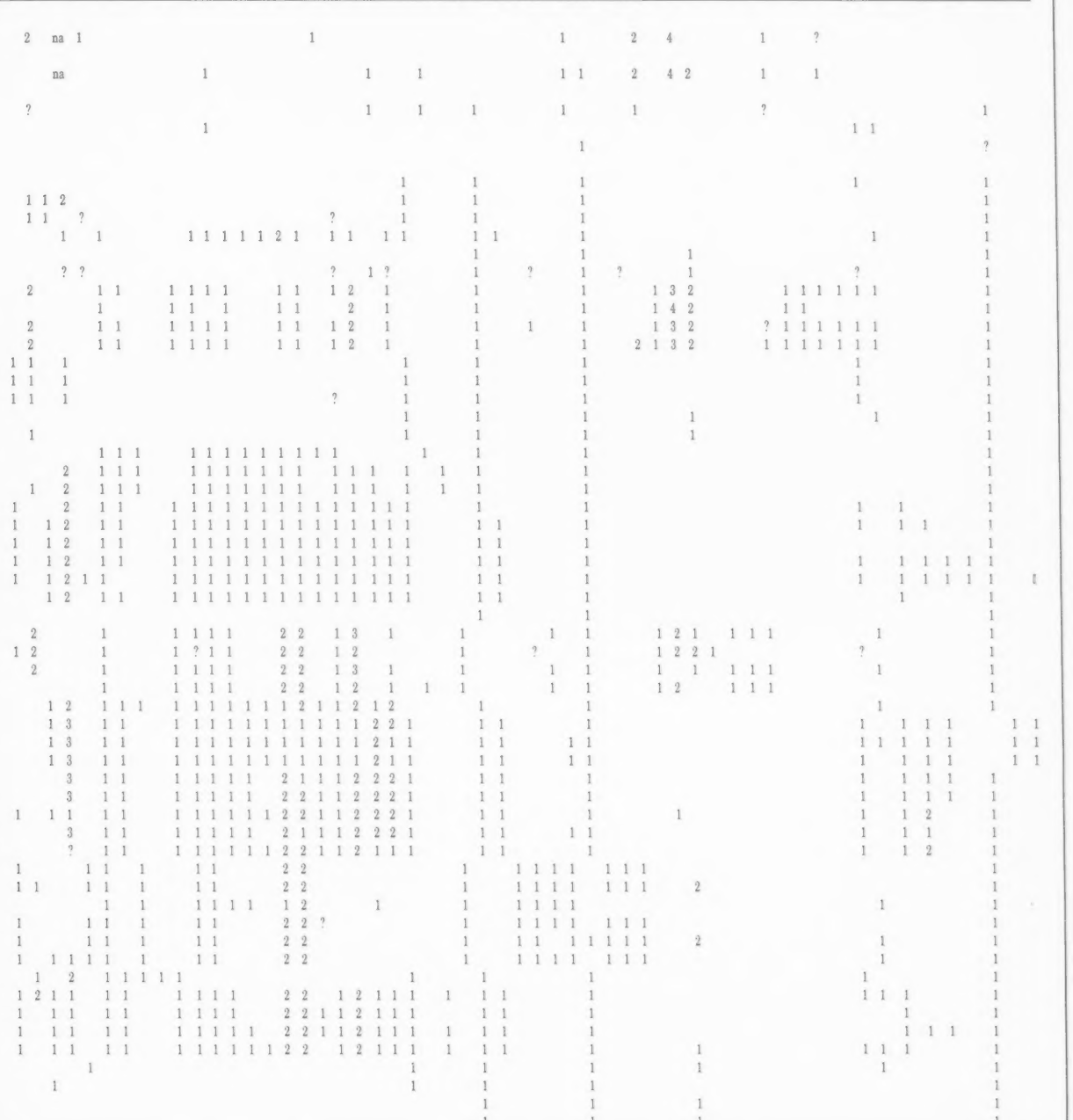

**Table 2**

Heuristic search results for an analysis of 106 characters for 53 ingroup taxa.

| Addition sequence | Branch-swapping algorithm | Tree length (steps) | Number of trees found | Cumulative total of unique trees |
|---|---|---|---|---|
| as is | tbr | 403 | 112 | 112 |
| as is | spr | 403 | 80 | 112 |
| as is | nni[1] | 405 | 16 | 128 |
| closest | tbr | 403 | 16 | 128 |
| closest | spr | 403 | 16 | 128 |
| closest | nni | 403 | 16 | 128 |
| simple | tbr | 403 | 112 | 128 |
| simple | spr | 403 | 16 | 128 |
| simple | nni[1] | 409 | 48 | 128 |
| random[2] | tbr | 403 | 128 | 128 |
| random[2] | spr | 403 | 128 | 128 |
| random[2] | nni | 403 | 128 | 128 |

[1] Trees found by this method were not of minimum length and not added to the total number of trees observed.
[2] One hundred replicates for each random addition sequence.

quences using the "nearest neighbour interchange" branch-swapping were the only combinations that did not find trees with 403 steps (Table 2). The results with 100 "random" addition replicates were not different from those observed with the three other methods (Table 2). This nonrigorous test suggests that heuristic search combinations were effective in finding all of the most parsimonious cladograms.

A quantitative estimate of data decisiveness (Goloboff, 1991) is not possible for an analysis of 53 taxa which requires calculation of mean length for all possible cladograms. However, a generalized statement of data decisiveness suggests that data for this analysis are decisive. "Data are strongly decisive if one or more cladograms explaining them is very much shorter than others, and only weakly decisive if all possible cladograms are not very different for each other in length" (Forey et al., 1992). In total, only 128 unique cladograms trees were observed (Table 2), which represents a minute fraction of possible trees, and only a slightly larger fraction of those actually examined during the search. Assuming that all trees of 403 steps were found, there must be many trees that have more than 403 steps. Although the frequency distribution of trees with number of steps cannot be determined, it is assumed that the number of trees with more than 403 steps must increase dramatically given that the maximum number of steps is 1384.

The majority-rule consensus of these trees (Fig. 1) illustrates clades observed in 50% or more of the 128 results. Clades found in less than 100% of the trees are indicated in parentheses as the percent of trees observed at this node. Only 5 of 47 resolved nodes were observed in less than 100% of the trees and only 2 were observed in less than 75% of the trees. Examination of character distribution in the tree reveals the homoplastic nature of many characters used in the analysis. This is reflected by the low consistency index (ci=0.33) observed in the 128 most parsimonious cladograms (Rohlf, 1982).

Low consistency index is expected in studies of interrelationships for large numbers of taxa (Sanderson and Donoghue, 1989). The expected consistency index for a study of 53 taxa would be 0.14 with an equation of linear regression derived from 60 previous cladistic studies (Sanderson and Donoghue, 1989). This consistency index suggests that there is less homoplasy describing the interrelationships of the Pleuronectidae than in other studies of this size. The retention index (ri=0.79) indicates that homoplasy is occurring at terminal nodes and not internal nodes, which, in turn, suggests that relationships at the level of subfamily, tribe, and genera are not based purely on homoplastic morphologies (Forey et al., 1992) and that the strength of this analysis can be used to determine relationships at this level. The retention index also suggests that this analysis is not effective at determining relationships near terminal ends, such as interrelationships among species within a genus. Consequently, the character analysis is restricted to the level of subfamily and tribe, and intragenera analysis will be explored only for relationships well corroborated by uniquely derived character states.

## Monophyly of the Pleuronectidae

Ten synapomorphies define the Pleuronectidae. Distributions of these states were also surveyed in the literature for taxa within the bothoid lineage (Hensley and Ahlstrom, 1984) and basal lineages within Pleuronectiformes (Chapleau, 1993).

1 Ocular-side frontal articulated with mesethmoid. Outgroup taxa with ocular-side prefrontal separating frontal from mesethmoid. Distribution of this structure within other pleuronectiform taxa reveals that the frontal on the ocular side is not articulated with the mesethmoid in Psettodidae, Citharidae, Paralichthyidae, Taeniopsettinae, and some genera of Bothinae (*Arnoglossus, Psettina, Asterorhombus, Japonolaeops, Laeops, Komoharaia, Neolaeops*, and *Chascanopsetta)* and is observed to be in contact with the mesethmoid only in the Bothinae genera (*Parabothus,*

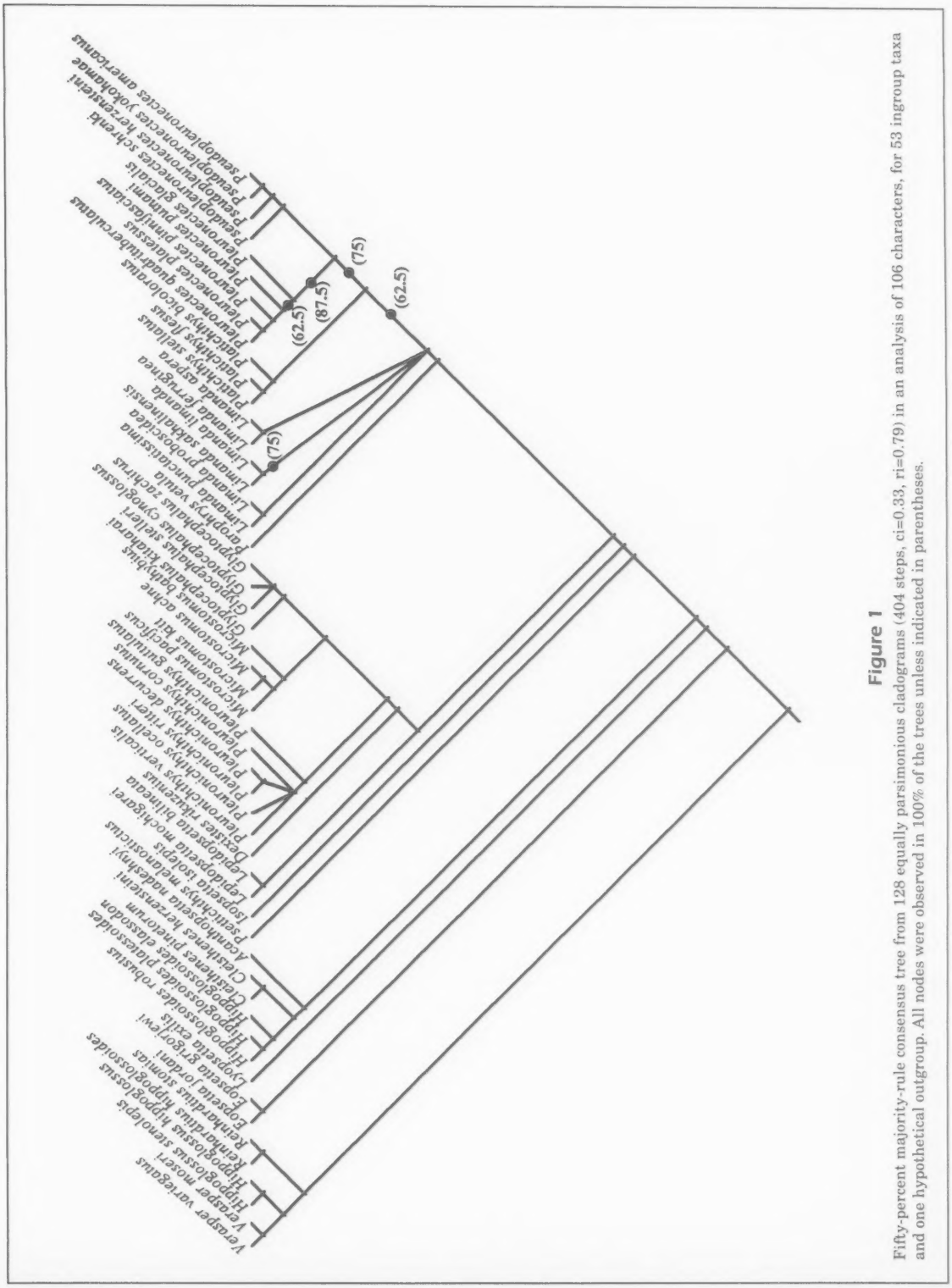


**Figure 1**

Fifty-percent majority-rule consensus tree from 128 equally parsimonious cladograms (404 steps, ci=0.33, ri=0.79) in an analysis of 106 characters, for 53 ingroup taxa and one hypothetical outgroup. All nodes were observed in 100% of the trees unless indicated in parentheses.

*Engyprosopon, Tosarhombus, Crossorhombus*, and *Bothus* [Amaoka, 1969]).

2 Ocular-side preorbital sensory canal absent, with exceptions only in *Reinhardtius hippoglossoides* and *Acanthopsetta nadeshnyi*. In the bothoid group, as well as in the Citharidae and Psettodidae, this sensory canal is present (Amaoka, 1969).

3 Ventral margin of metapterygoid flattened (Fig. 2, B–D), with exception in *Reinhardtius stomias*, which has a distinct curvature along the ventral margin of this bone. Outgroup taxa also possess this ventral curvature of the metapterygoid (Fig. 2A).

4 First and second basibranchials loosely joined by cartilage with exceptions observed in *Eopsetta grigorjewi*, *Isopsetta isolepis*, *Limanda ferruginea*, *L. punctatissima*, *Parophrys vetula*, *Psettichthys melanostictus*, and *Reinhardtius stomias* in which basibranchials are sutured. Outgroup taxa also have a suture between first and second basibranchials (Fig. 3A).

5 Second and third basibranchial loosely joined by cartilage (Fig. 3, B–D) with exceptions in *Limanda punctatissima* and *Psettichthys melanostictus*. Outgroup taxa have a suture between second and third basibranchials (Fig. 3A).

6 Posteriormost abdominal vertebrae lack haemapophysis (Fig. 4C), with exceptions in *Eopsetta grigorjewi*, *Microstomus achne*, *M. kitt*, *M. pacificus*, and *Reinhardtius stomias* in which haemapophysis are present (Fig. 4D). Outgroups have fused parapophysis forming a haemal arch on the posteriormost abdominal vertebrae (Fig. 4, A and B).

7 Accessory processes on caudal vertebrae absent (Fig. 5, B and C), with exceptions in *Hippoglossus hippoglossus*, *H. stenolepis*, *Microstomus bathybius*, *Pleuronichthys decurrens*, *P. guttulatus*, and *P. ritteri*. Outgroups have accessory processes on ventral surface of centrum for all caudal vertebrae (Fig. 5A).

8 Ocular-side infraorbital bones present with exception in *Microstomus bathybius*. Outgroup taxa, except *Psettodes*, do not have infraorbital bones on the ocular side. *Psettodes* has four infraorbital bones on the ocular side. Presence of infraorbital bones in Pleuronectidae, although not unique in comparison with all outgroup taxa, is likely a reversal in the bothoid lineage and synapomorphic for the family.

9 Oil globules in egg absent, with exceptions of one oil globule found in *Pleuronichthys cornutus, P. guttulatus*, and *P. ritteri* (Ahlstrom et al., 1984). Outgroups have at least one oil globule (Ahlstrom et al., 1984). Distribution of this character was not confirmed in the specimens used for this analysis; however the source for this information (Ahlstrom et al., 1984) confirms this distribution in 46 of the 59 pleuronectid species.

10 Olfactory laminae are parallel without a central rachis, with exception in *Reinhardtius evermanni* and *R. stomias*. As in the outgroup, species of *Reinhardtius* have laminae that radiate from a central rachis (Norman, 1934). Distribution of this structure was not confirmed in this analysis. Historically, this structure has been used to diagnose the Pleuronectinae (*sensu* Norman, 1934) and appears to be unique in flatfishes. There has not been any evidence to suggest that this occurs in any other flatfish species.

Exceptions in distribution of these ten synapomorphies do not have a common phylogenetic pattern and do not corroborate exclusion of any of the 53 species examined in this analysis. The exceptions suggest independent cases of reversed characters (reversals) for either the species or their immediate ancestors, and these will be discussed in the context of species interrelationships within the Pleuronectidae. It is noted that *Reinhardtius stomias* has four exceptions to the ten synapomorphies in Pleuronectidae. The most parsimonious explanation places this species in the Hippoglossinae on the basis of seven additional synapomorphies. Other characters (48, 77, 83, and 104) appear to be synapomorphies for the Pleuronectidae. However, distribution of these characters was examined in only the 53 ingroup taxa and in the five outgroup taxa used for this analysis. This limited survey is not sufficient to provide a full understanding of the distribution of these character states within the order. Therefore these character states are not presented as synapomorphies for the family and are only presented in a phylogenetic context within Pleuronectidae. Future analysis examining higher-level relationships within Pleuronectiformes should include these morphological characters.

## Intrarelationships of the Pleuronectidae

The phylogenetic analysis reveals various monophyletic lineages within the Pleuronectidae, four of which illustrate the interrelationships of five newly defined subfamilies: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae (Fig. 6). These subfamilies are separated by a gradation of characters (11 to 22) such that the first three, Hippoglossinae, Eopsettinae, and Lyopsettinae, contain species with a large proportion of plesiomorphic

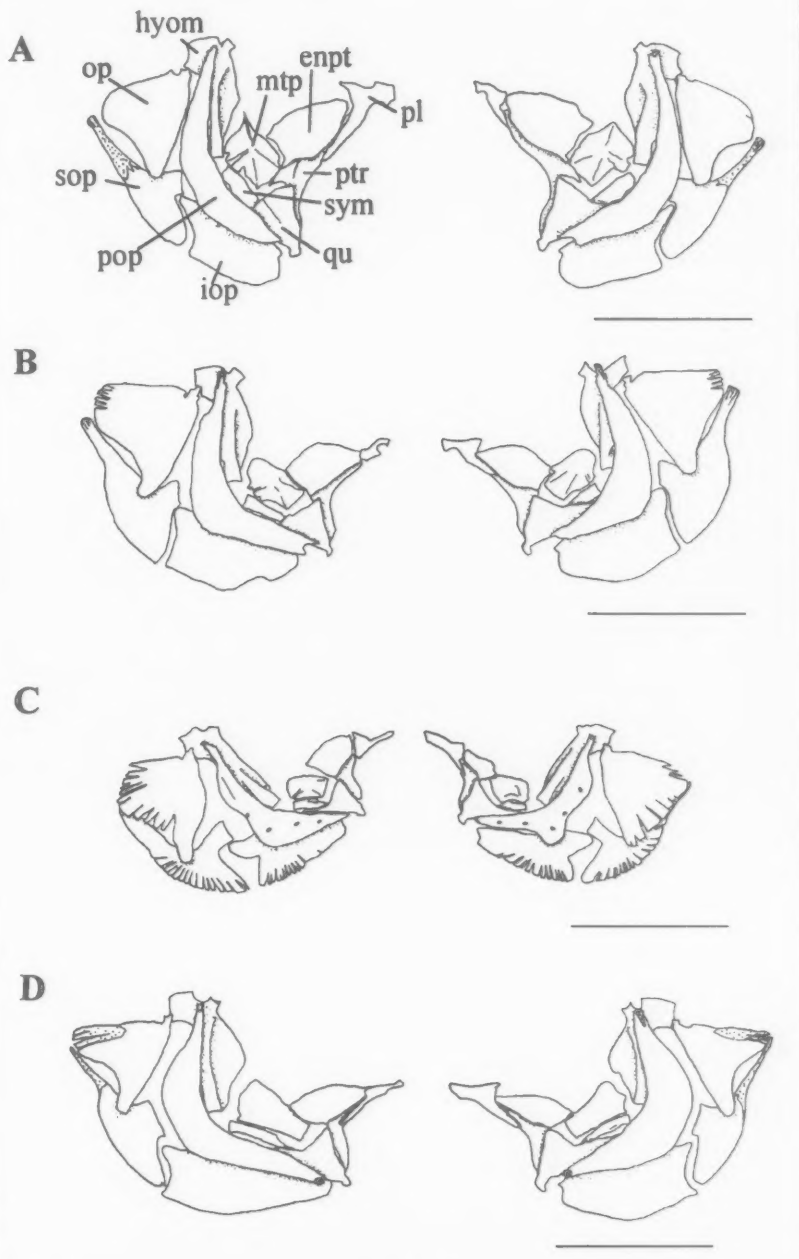


**Figure 2**

Lateral aspect of suspensorium. (Left) ocular side; (right) blind side. (**A**) *Paralichthys lethostigmus*, ANSP 143209; (**B**) *Verasper moseri*, USNM 056385; (**C**) *Microstomus pacificus*, NMC 81-1027; (**D**) *Limanda ferruginea*, NMC 80-0217. enpt = entopterygoid; hyom = hyomandibular; iop = interoperculum; mtp = metapterygoid; op = operculum; pl =palatine; pop = preoperculum; ptr = pterygoid; sop = suboperculum; sym = symplectic; qu = quadrate. Scale bars are 10 mm.

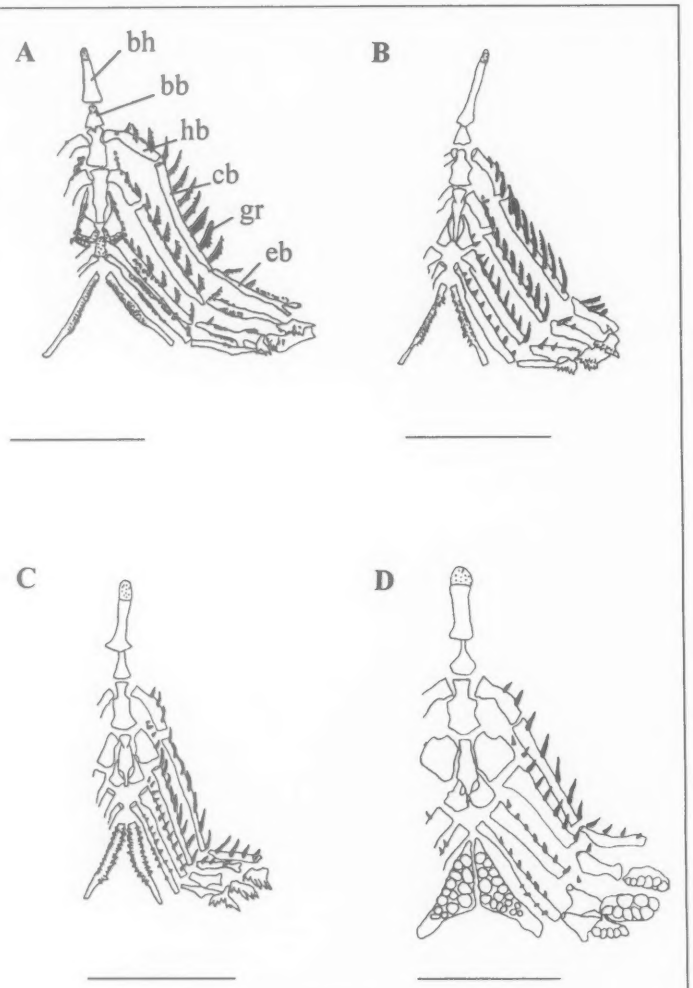


**Figure 3**

Dorsal aspect of branchial apparatus. (**A**) *Paralichthys lethostigmus*, ANSP 143209; (**B**) *Lyopsetta exilis*, NMC 60-0501; (**C**) *Limanda ferruginea*, NMC 80-0217; (**D**) *Pleuronectes putnami*, ROM 556. bb=basibranchial; bh = basihyal; cb = ceratobranchial; eb = epibranchial; gr = gill raker; hp = hypobranchial. The fourth basibranchial is cartilagenous and illustrated in only Fig. 3A. Scale bars are 10 mm.

character states. As a result, interrelationships within these groups are supported by few synapomorphies. Serving as successive outgroups (Stiassny and de Pinna, 1994), the position of these three subfamilies determines the polarity and relationships within the Hippoglossoidinae and the diversified Pleuronectinae.

The first lineage (I) distinguishes Hippoglossinae from all other pleuronectid taxa. The second lineage (II) contains all species classified in Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae. This second lineage is supported by two synapomorphies (Fig. 6): ocular-side pterosphenoid and prootic join to form the dorsal margin of anterior prootic foramen (11, Fig. 7, B and C); and first epibranchial not bifurcated at its distal end (12, Fig. 3, C and D).

Exceptions to the distribution of these two characters are observed in Pleuronectinae and *Verasper*

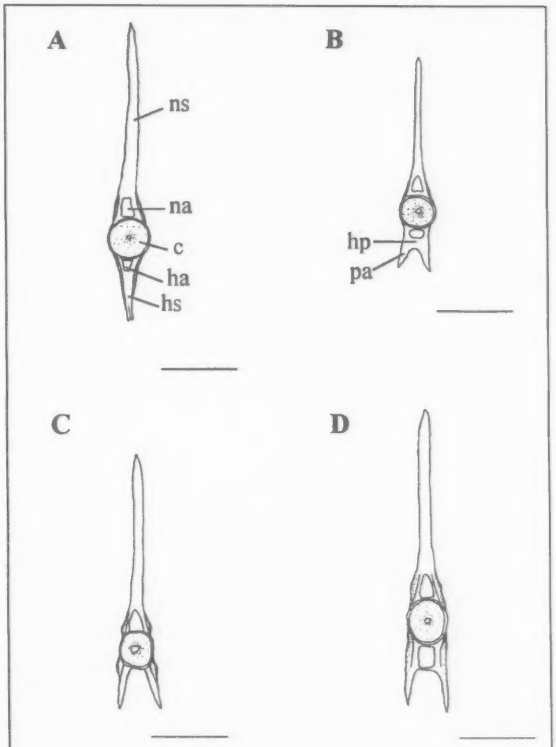


**Figure 4**

Cranial aspect of abdominal vertebrae. (**A**) *Lepidoblepharon ophthalmolepis*, AMS I.20118-012; (**B**) *Paralichthys lethostigmus*, ANSP 143209; (**C**) *Hippoglossoides robustus*, ANSP 105133; (**D**) *Microstomus kitt*, FMNH 355327. c = centrum; ha = haemal arch; hp = haemapophysis; hs = haemal spine; na = neural arch; ns = neural spine; pa = parapophysis. Scale bars are 5 mm.

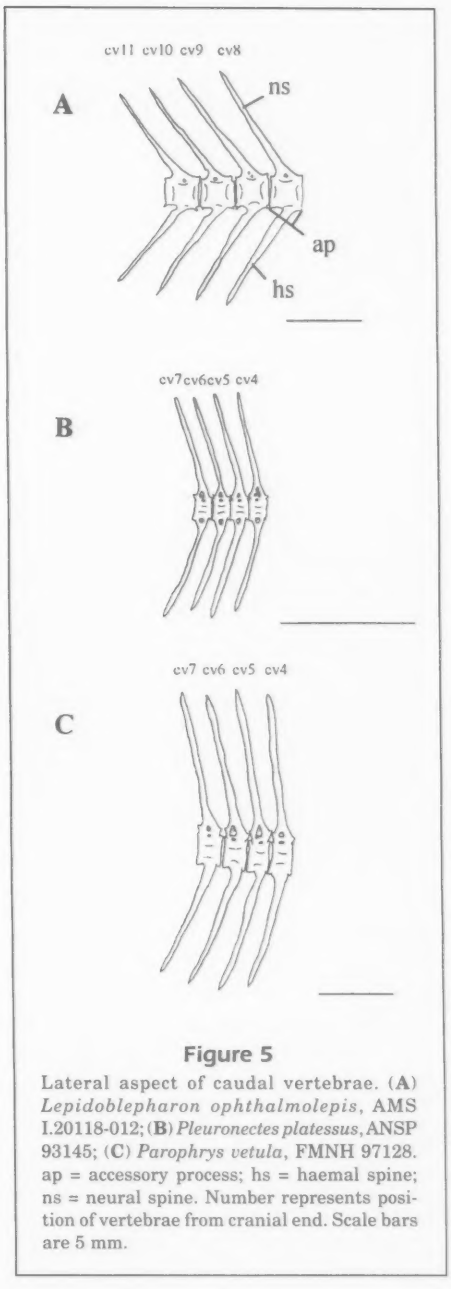


**Figure 5**

Lateral aspect of caudal vertebrae. (**A**) *Lepidoblepharon ophthalmolepis*, AMS I.20118-012; (**B**) *Pleuronectes platessus*, ANSP 93145; (**C**) *Parophrys vetula*, FMNH 97128. ap = accessory process; hs = haemal spine; ns = neural spine. Number represents position of vertebrae from cranial end. Scale bars are 5 mm.

(Hippoglossinae). The pterosphenoid and prootic do not unite to form the dorsal margin of the anterior prootic foramen (Fig. 7A) in the following pleuronectine species: *Glyptocephalus cynoglossus*, *G. stelleri*, *G. zachirus*, *Limanda ferruginea*, *L. proboscidea*, *L. punctatissima*, *Platichthys stellatus*, *Pleuronichthys guttulatus*, and *Pseudopleuronectes americanus*. *Verasper variegatus*, not classified in this second lineage, has the dorsal margin of this foramen formed by the pterosphenoid and prootic on both ocular and blind side. The first epibranchial is bifurcated in the pleuronectine species *Dexistes rikuzenius*, *Limanda aspera*, *L. limanda*, *L. sakhalinensis*, *Glyptocephalus*, *Microstomus*, and *Pleuronichthys*. Both species of *Verasper* were observed to have a bifurcated first epibranchial. The pattern of these exceptions is similar in these two characters, but the analysis did not indicate an alternative topology that would exclude any of the previously mentioned species, or include *Verasper* within the second lineage. The pattern does suggest that these exceptions are instances of reversal or convergence and may determine phylogeny within these other groups.

The third pleuronectid lineage (III) indicates a common ancestor for Lyopsettinae, Hippoglossoidinae,

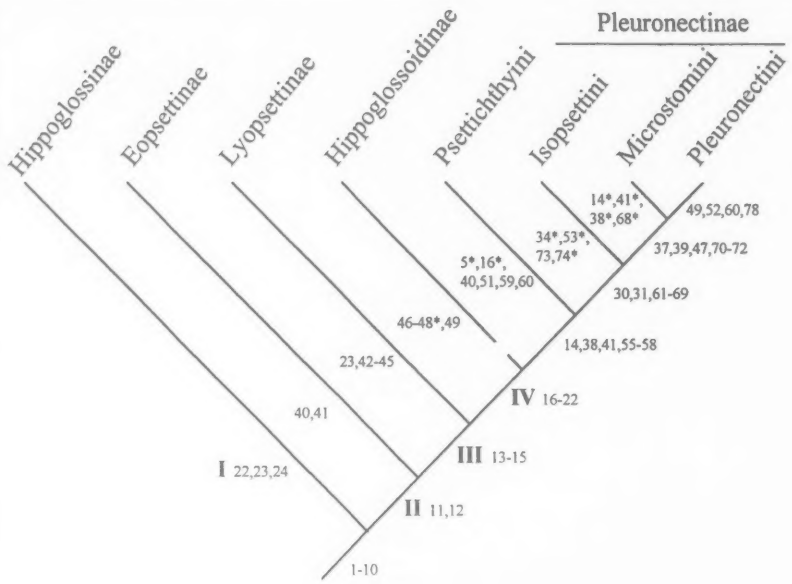


**Figure 6**

Intrarelationship for subfamilies and tribes in Pleuronectidae. Numbers correspond to characters in text. Asterisk represent character state reversal.

and Pleuronectinae. Three synapomorphies defining this lineage (Fig. 6) are spines absent on gill rakers (13, Fig. 3, C and D); anterior margin of upper orbit complete with an overlap between mesethmoid and prefrontal of the blind side (14, Fig. 8, C and D); and first anal pterygiophore broadly thickened (15).

Exceptions to the distribution in these synapomorphies are found in Pleuronectinae and Hippoglossinae. The anterior margin of the upper orbit is incomplete in *Microstomus achne, M. bathybius, M. kitt, M. pacificus, Pleuronectes pinnifasciatus, Pseudopleuronectes americanus, P. herzensteini*, and *P. yokohamae. Reinhardtius stomias,* which also has the derived state for this character, is excluded from this third lineage. The first anal pterygiophore is not thickened in *Microstomus achne, M. bathybius, M. kitt*, and *M. pacificus*, whereas in *Hippoglossus* and *Verasper* (Hippoglossinae) the first anal pterygiophore is broadly thickened.

The fourth lineage (IV) includes all species of Hippoglossoidinae and Pleuronectinae. The sister relationship between these two subfamilies is determined by seven synapomorphies (Fig. 6): dentition of uniform size (16, Fig. 9, B–D); interorbital process reduced or completely absent (17, Fig. 10, B, D, and E); hyomandibula broadened anteriorly (18, Fig. 2D); dentition on third epibranchial absent (19, Fig. 3, C and D); bony plates absent on branchial arches (20, Fig. 3, C and D); two rows of teeth present on fourth ceratobranchial (21, Fig. 3, C and D); and dorsoposterior margin of operculum fimbriated (22, Fig. 2, B–D).

Exceptions to these character distributions are found in only two species of *Hippoglossoides* and in Microstomini. *Hippoglossoides platessoides* and *H. robustus* have larger anterior teeth (16) that were historically termed as "canines" (Norman, 1934). *Glyptocephalus kitaharai, G. zachirus, Microstomus achne, M. kitt, M. pacificus*, and all species in *Pleuronichthys* have an interorbital process (17, Fig. 10C). The anterior margin of the hyomandibular (18) is not broadened in *Glyptocephalus* and *Microstomus* (Fig. 2C). Dentition on the third epibranchial and bony plates on branchial arches are observed in *Pleuronichthys guttulatus*. The number of rows of teeth on the fourth ceratobranchial are reduced to only one in *Glyptocephalus*, *Platichthys bicoloratus*, and *Pleuronichthys* and are absent in *Limanda punctatissima*. Fimbriation of the operculum (22) is also observed in Hippoglossinae (Fig. 2B) but is absent in the Eopsettinae and Lyopsettinae. This last exception suggests that fimbriation of the operculum may be synapomorphic for Pleuronectidae because only *Eopsetta grigorjewi*, *E. jordani*, and *Lyopsetta*

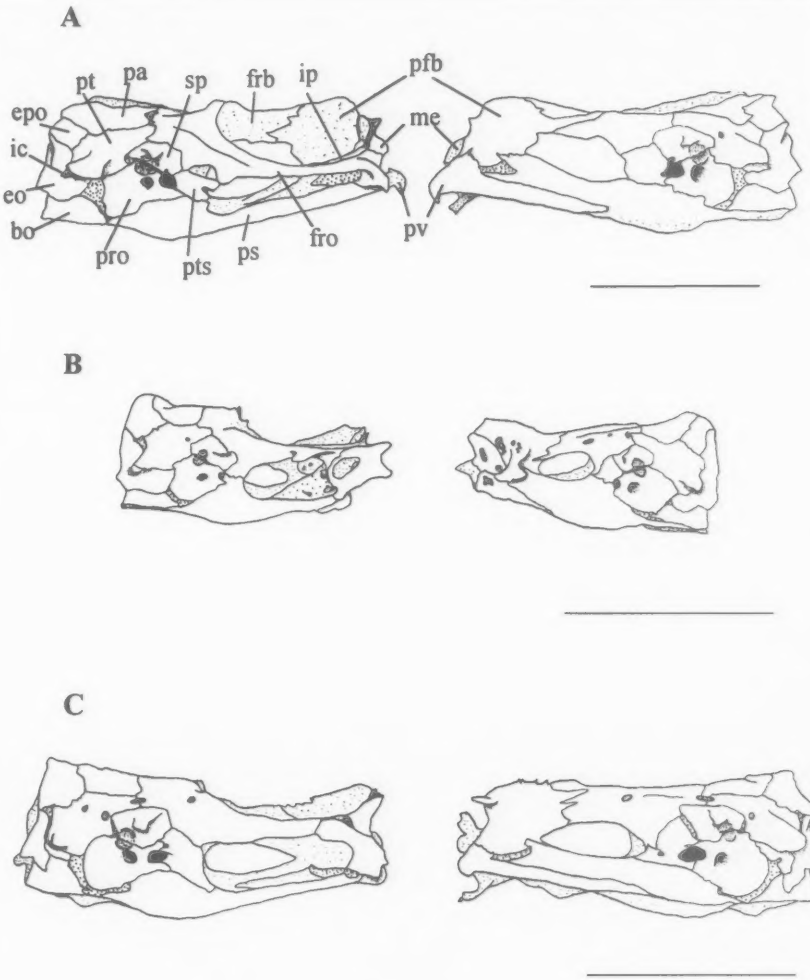


**Figure 7**

Lateral aspect of crania. (**A**) *Hippoglossus hippoglossus*, ARC 8808487; (**B**) *Pleuronichthys cornutus*, UMMZ 159618; (**C**) *Limanda limanda*, MNHN 1959-560. Left is ocular side; right is blind side. bo = basioccipital; eo = exoccipital; epo = epiotic; frb = frontal (blind side); fro = frontal (ocular side); ic = intercalar; ip = interorbital process; me = mesethmoid; pa = parietal; pfb = prefrontal (blind side); pro = prootic; ps = parasphenoid; pt = pterotic; pts = pterosphenoid; pv = prevomer; sp = sphenotic. Scale bars are 10 mm.

*exilis* do not show this condition. This alternative hypothesis would require three evolutionary steps, one more than is presently hypothesized. An equivocal alternative (two steps) would require a single reversal in character 22 to define a monophyletic group of *Eopsetta* and *Lyopsetta*. However, this topology was not observed in any of the 128 most parsimonious trees.

**Subfamily Hippoglossinae** The first pleuronectid lineage is classified as Hippoglossinae, with eight species (6 examined) in four genera: *Clidoderma* (*incertae sedis*), *Hippoglossus*, *Reinhardtius*, and *Verasper* (Fig. 11). This subfamily, as well as the intrarelationships of its species, were observed in all of the most parsimonious cladograms. The Hippoglossinae are hypothesized to be monophyletic ac-

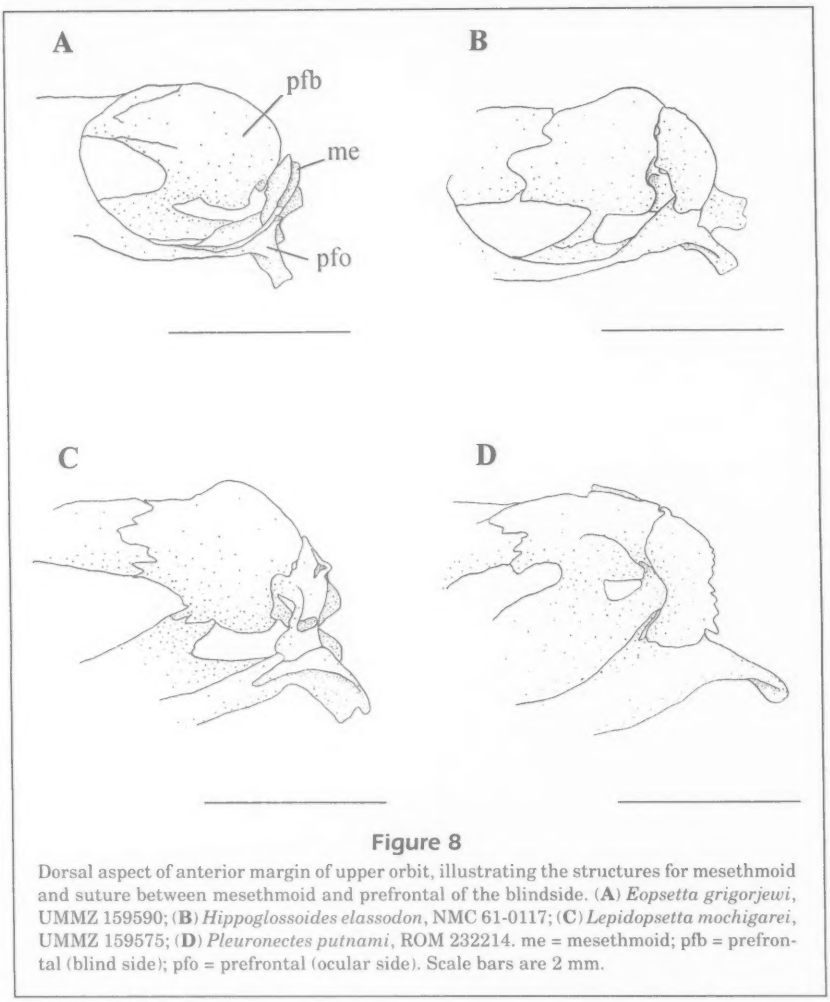


**Figure 8**

Dorsal aspect of anterior margin of upper orbit, illustrating the structures for mesethmoid and suture between mesethmoid and prefrontal of the blindside. (**A**) *Eopsetta grigorjewi*, UMMZ 159590; (**B**) *Hippoglossoides elassodon*, NMC 61-0117; (**C**) *Lepidopsetta mochigarei*, UMMZ 159575; (**D**) *Pleuronectes putnami*, ROM 232214. me = mesethmoid; pfb = prefrontal (blind side); pfo = prefrontal (ocular side). Scale bars are 2 mm.

cording to three synapomorphies (Fig. 6): increase in number of abdominal vertebrae to more than 12 (23); lunate-shaped caudal fin (24); and presence of fimbriation on dorsoposterior opercular margin (22, Fig. 2B). These characters are found to have a high degree of homoplasy within the Pleuronectidae. The fimbriation pattern of the opercular margin (22) appears in all other species of Pleuronectidae, except in *Eopsetta* and *Lyopsetta* that comprise the next two lineages. An increase in the number of abdominal vertebrae (23) is also observed in other subfamilies and the lunate-shaped caudal fin (24) is not observed in all members of this lineage; a reversal is hypothesized for *Verasper*. Monophyletic status of this clade is suspect. The low proportion of derived character states inherent for basal lineages provides little support for monophyly and intrarelationships for these taxa. Analysis of morphological characters that are homoplastic at the familial level, but not so at lower levels of universality, might clarify both the monophyletic status of this subfamily and its intrarelationships.

### Intrarelationships of Hippoglossinae

**Genus *Reinhardtius*** This genus contains three species: *R. hippoglossoides*, *R. evermanni* (not examined), and *R. stomias*, characterized by four synapomorphies (Fig. 11): gill rakers absent on fourth ceratobranchial (21); migrated eye is situated near dorsal midline of cranium, so that it is visible from blind side (25); more than 35 caudal vertebrae (26); and dentary foramen absent on blind side (27).

Homoplasy observed for these characters is observed in unrelated pleuronectid taxa and does not

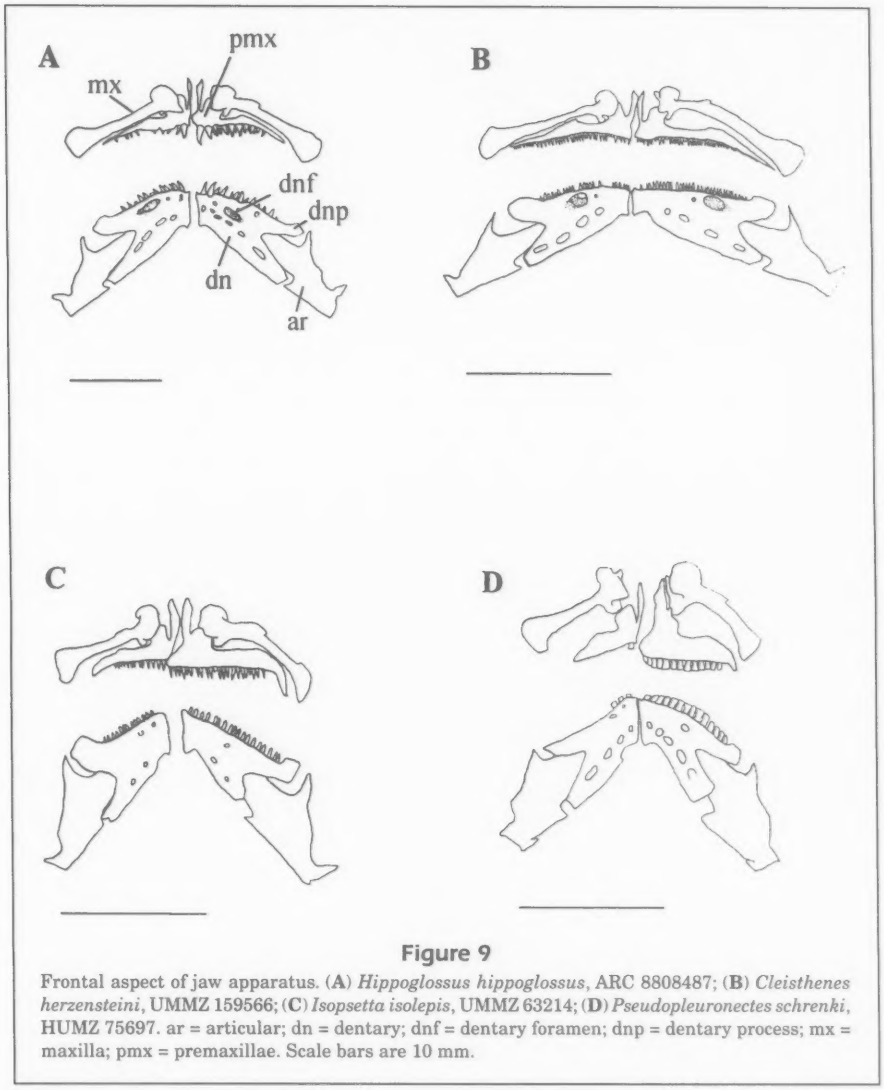


**Figure 9**

Frontal aspect of jaw apparatus. (**A**) *Hippoglossus hippoglossus*, ARC 8808487; (**B**) *Cleisthenes herzensteini*, UMMZ 159566; (**C**) *Isopsetta isolepis*, UMMZ 63214; (**D**) *Pseudopleuronectes schrenki*, HUMZ 75697. ar = articular; dn = dentary; dnf = dentary foramen; dnp = dentary process; mx = maxilla; pmx = premaxillae. Scale bars are 10 mm.

corroborate an alternative hypothesis. The absence of gill rakers on fourth ceratobranchial is observed in *Limanda punctatissima* (Pleuronectinae). The position of the migrated eye in relation to the dorsal midline is a reversal also observed in *Cleisthenes pinetorum* (Hippoglossoidinae). An increase in the number of caudal vertebrae is also observed in *Glyptocephalus* and *Microstomus,* a clade of nine species whose monophyletic status is strongly supported within the Pleuronectinae. Absence of the dentary foramen on the blind side is only observed in four other pleuronectid species including *Hippoglossus hippoglossus*.

*Reinhardtius evermanni* was not examined, but morphological characters reported in the literature suggest monophyly for *R. evermanni*, *R. hippoglossoides*, and *R. stomias*. *Reinhardtius evermanni* also has an increased number of caudal vertebrae, ranging from 37 to 40 (Sakamoto, 1984a). *Reinhardtius evermanni* and *R. stomias* share two unique structures: olfactory lamellae that radiate from a central rachis; and jaw and pharyngeal teeth with barbed tips (Norman, 1934). If these structures are considered synapomorphic within the genus, then *Reinhardtius evermanni* and *R. stomias* are sister species, with *R. hippoglossoides* immediately basal to them.

The sister relationship between *Hippoglossus* and *Verasper* is supported by six synapomorphies (Fig. 11):

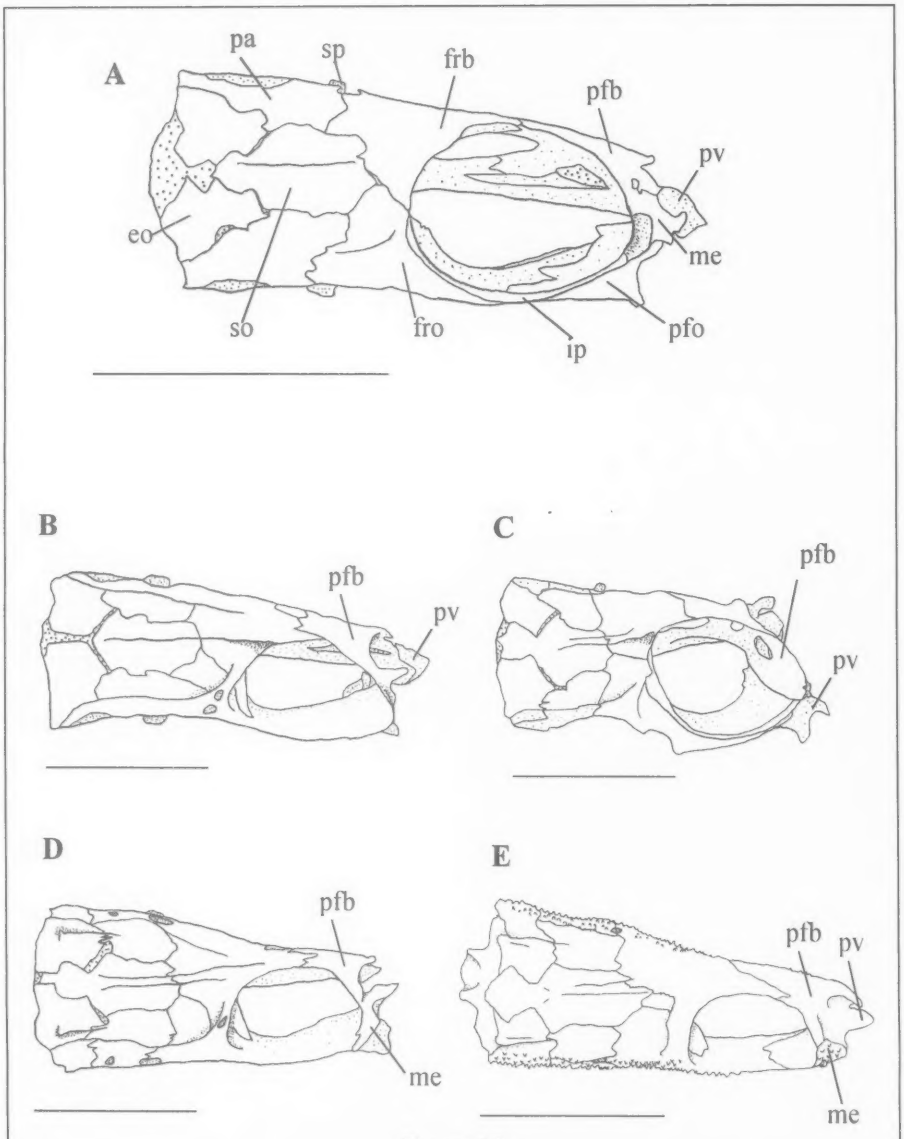


**Figure 10**

Dorsal aspect of crania. (**A**) *Reinhardtius stomias*, NMC 81-1024; (**B**) *Hippoglossoides robustus*, ANSP 105133; (**C**) *Pleuronichthys ocellatus*, CAS 82189; (**D**) *Parophrys vetula*, FMNH 97128; (**E**) *Limanda punctatissima*, HUMZ 93958. eo = exoccipital; frb = frontal (blind side); fro = frontal (ocular side); ip = interorbital process; me = mesethmoid; pa = parietal; pfb =prefrontal (blind side); pfo = prefrontal (ocular side); pv = prevomer; so = supraoccipital; sp = sphenotic. Scale bars are 10 mm.

first anal pterygiophore broadly thickened (15); ocular-side palatine reduced and not articulated with pterygoid (28, Fig. 2B); gill rakers on first epibranchial absent (29) with exception in *Hippoglossus stenolepis*; gill rakers on second and third epibranchials (30, 31) reduced (*Hippoglossus*) or absent (*Verasper*); and gill rakers on first hypobranchial reduced (32).

These states are homoplastic in other lineages of the Pleuronectidae. Thickening of the first anal pterygiophore (15) unites taxa in the third pleuronectid lineage. Reduction of the palatine on the ocu-

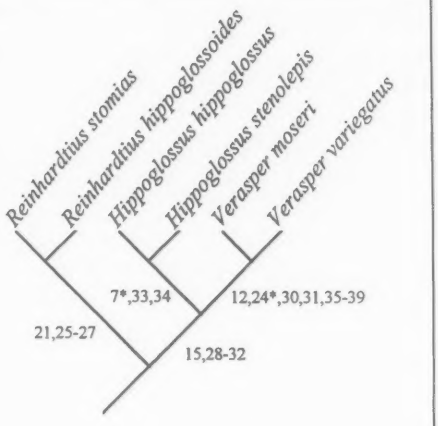


**Figure 11**

Intrarelationships of Hippoglossinae. Numbers correspond to characters in text. Asterisk represents character state reversal.

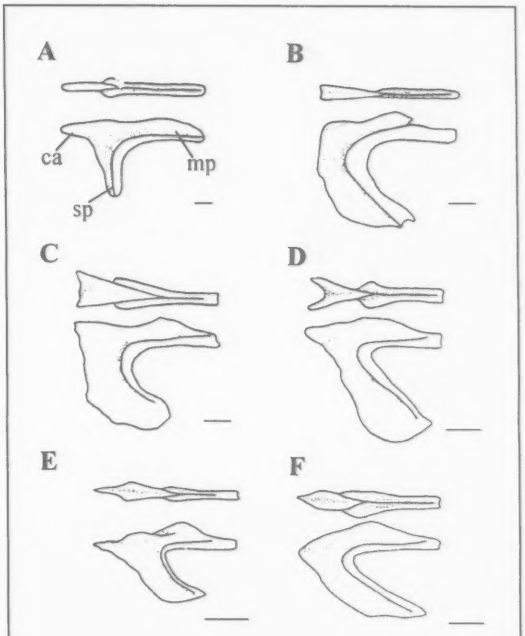


**Figure 12**

Dorsal (above) and lateral (below) aspects of urohyal (modified from Sakamoto, 1984a). (**A**) *Reinhardtius hippoglossoides* (**B**) *Hippoglossus stenolepis* (**C**) *Verasper variegatus* (**D**) *Pseudopleuronectes herzensteini* (**E**) *Glyptocephalus kitaharai* (**F**) *Dexistes rikuzenius*. ca = cardiac apophysis; mp = main part; sp = sciatic part. Scale bars are 2 mm.

lar side (28, Fig. 2, B and C) also defines the lineage uniting *Glyptocephalus*, *Microstomus*, and *Pleuronichthys* within the Pleuronectinae. Absence or reduction of gill rakers on first, second, and third epibranchials (29, 30, 31) is found to define basal lineages within the Pleuronectinae, whereas reduction of gill rakers on the first hypobranchial is observed in only some species of *Pleuronichthys* and *Platichthys bicoloratus*.

**Genus *Hippoglossus*** This genus contains two species: *H. hippoglossus* and *H. stenolepis* and is monophyletic with three synapomorphies (Fig. 11): presence of subdivisions in hypural and parahypural plates, an autapomorphy for the genus (33); presence of accessory processes on ventral margin of caudal vertebrae (7, Fig. 5A), a reversal for the family; and metapterygoid articulated with the blind-side entopterygoid (34, Fig. 2C). Only *Microstomus bathybius* and three species of *Pleuronichthys* have accessory processes on the caudal vertebrae. The metapterygoid is also articulated with the entopterygoid of the blind side in *Reinhardtius hippoglossoides* and species within Pleuronectinae.

**Genus *Verasper*** This genus, containing *V. moseri* and *V. variegatus* is monophyletic with nine synapomorphies (Fig. 11): presence of a large foramen between mesethmoid and blind-side prefrontal (35), autapomorphic for *Verasper*; first epibranchial bifurcated (12); caudal fin is rounded (24), a reversal in this subfamily; gill rakers absent on both second and third epibranchials (30, 31); gill rakers reduced on second hypobranchial (36); sphenotic process not forming dorsal margin of hyomandibular socket (37, Fig. 7, B and C); groove present along supraoccipital crest for insertion of pterygiophores (38, Fig. 10, C–E); and cardiac apophysis of urohyal bifurcated (39, Fig. 12C).

Many character states found within *Verasper* are also observed in the Pleuronectinae, but the strength of the hypothesis placing *Verasper* within Hippoglossinae exceeds the characters mentioned above and illustrates the convergent evolution of these structures.

**Genus *Clidoderma*** *C. asperrimum* was not available for analysis. This species has modified scales on the ocular side to form distinct bony tubercles very similar to those observed in *Platichthys*. However, this unique species appears to be more closely related to *Verasper* than to *Platichthys* (Norman, 1934). This species has subsymmetrical jaws and a mix of pointed and bluntly conical teeth, not uniform in length, set in multiple rows on both upper and lower jaws. These features are plesiomorphic for the fam-

ily and only exclude *Clidoderma* from the fourth pleuronectid lineage. This species has 14 abdominal vertebrae found to be synapomorphic for Hippoglossinae; it also has a thickened first anal pterygiophore suggesting a common ancestry with *Hippoglossus* and *Verasper*. A rounded caudal fin, observed in *Verasper* is also observed in *Clidoderma*. Phylogenetic position of this species could be further ascertained by an examination of the accessory processes on caudal vertebrae, the palatine structure, and the structure of gill rakers on first, second, and third epibranchials.

**Subfamily Eopsettinae** The Eopsettinae consists of *E. grigorjewi* and *E. jordani*. This analysis defines this subfamily with two synapomorphies (Fig. 6): presence of gill rakers on fourth epibranchial (40); and a single row of teeth on lower jaw (41). Gill rakers on the fourth epibranchial are observed in *Cleisthenes herzensteini*, *C. pinetorum* (Hippoglossoidinae), and *Psettichthys melanostictus* (Psettichthyini). The number of rows of teeth on the lower jaw were found to be much more homoplastic. The latter feature was also observed in *Reinhardtius hippoglossoides*, *Verasper moseri*, and *V. variegatus* (Hippoglossinae) as well as in many species of the Pleuronectinae.

**Genus *Eopsetta*** This genus was described in Norman (1934) by a number of plesiomorphic characters. The presence of distinct canines on the upper jaw was suggested in Norman (1934) as diagnostic for *Eopsetta*. However, distribution of this character in the Pleuronectidae is not well defined. Members of the Hippoglossinae also have teeth of irregular lengths (16, Fig. 9A). The longer teeth can also be interpreted as canines in these species. From this analysis, data supporting monophyly of *Eopsetta* are not conclusive, but no other interpretation is available owing to insufficient information.

**Subfamily Lyopsettinae**

**Genus *Lyopsetta*** This lineage contains only *L. exilis*. Its position as a monotypic lineage within the Pleuronectidae is determined by five derived character states (Fig. 6): 12 to 14 abdominal vertebrae (23); barbed teeth present on dentaries and premaxillae (42); supratemporals on both ocular and blind sides are jointed at anterior ends of their bifurcation (43, 44, Fig. 13B); and presence of scales on eye surfaces (45).

These structures are also distributed within other pleuronectid taxa. An increase in abdominal vertebrae is found in Hippoglossinae, some species of Hippoglossoidinae, and in two separate lineages of Pleuronectinae. Barbed teeth are also observed in *Reinhardtius*. Bifurcation of the supratemporals is also observed in *Reinhardtius hippoglossoides*, *Cleisthenes*, *Hippoglossoides*, *Glyptocephalus cynoglossus*, *G. stelleri*, *G. zachirus*, *Microstomus pacificus*, *Limanda aspera*, and *Pleuronectes platessus*. Presence of scales on the eye surfaces is also found in *Reinhardtius stomias*, *Acanthopsetta nadeshnyi*, *Dexistes rikuzenius*, *Glyptocephalus kitaharai*, and *Microstomus*. The distribution of these character states within the family does not indicate an alternative hypothesis of relationships between *Lyopsetta exilis* and other pleuronectid taxa.

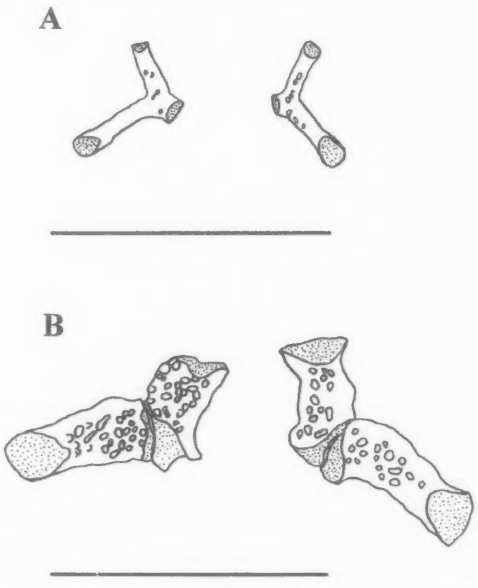


**Figure 13**
Lateral aspect of supratemporal. (**A**) *Verasper moseri*, USNM 056385; (**B**) *Limanda aspera*, NMC 66-0016. Left, ocular side; right, blind side. Scale bars are 5 mm.

**Subfamily Hippoglossoidinae** This subfamily contains seven species (6 examined) in three genera: *Acanthopsetta, Cleisthenes,* and *Hippoglossoides*. This group is characterized by four synapomorphies (Fig. 6): absence of supraoccipital plate extending posteroventrally between epiotics (46, Fig. 10B); pterosphenoid and prootic of blind side join to form dorsal margin of anterior prootic foramen (47, Fig. 7, B and C); pterosphenoid of blind side is reduced and does not form posterior margin of orbit (48, Fig. 7B), a reversal, except in *Cleisthenes;* and two uniform rows of teeth present on fifth ceratobranchial (49).

Distribution of these states within other pleuronectid taxa indicates a degree of homoplasy for these characters but does not refute the monophyly of Hippoglossoidinae. The supraoccipital plate is also absent in *Microstomus* (Microstomini). Pterosphenoid and prootic of the blind side are also united to form the dorsal margin of the anterior prootic foramen in *Verasper variegatus* and in most species of the Pleuronectinae. Reduction of the blind-side pterosphenoid is also observed in *Pleuronichthys* (Microstomini) and may be a reversal within the Pleuronectidae. Two rows of uniform teeth are also present in *Dexistes rikuzenius* (Microstomini) and the Pleuronectini.

### Intrarelationships of Hippoglossoidinae

**Genus *Acanthopsetta*** *A. nadeshnyi* is the sister species to *Cleisthenes* and *Hippoglossoides* (Fig. 14). Placement of *A. nadeshnyi* as a distinct lineage within Hippoglossoidinae is supported by two structures not present in other Hippoglossoidinae: sensory canal on ocular-side preorbital present (2); and presence of scales on eye surfaces (45). The presence of a sensory canal on the ocular-side preorbital is a reversal for the family that also occurs in *Reinhardtius hippoglossoides*. Scales present on the surface of each eye have a more homoplastic distribution as they are found in *Reinhardtius stomias, Lyopsetta exilis, Dexistes rikuzenius, Glyptocephalus kitaharai*, and *Microstomus*.

The sister relationship between *Cleisthenes* and *Hippoglossoides* is supported by three synapomorphies (Fig. 14): ocular-side supratemporal jointed at anterior end of bifurcation (43, Fig. 13B); nasal bones of the blind side are absent (50); and more than seven infraorbital bones on ocular side (51).

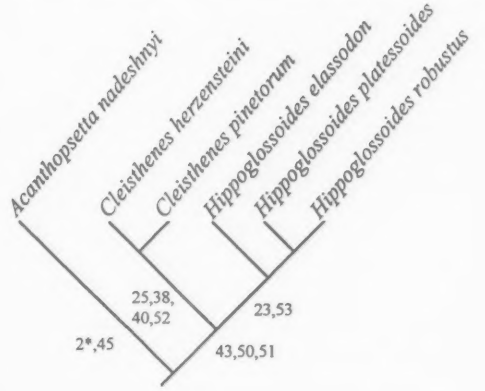


**Figure 14**

Intrarelationships of Hippoglossoidinae. Numbers correspond to characters in text. Asterisk represents character state reversal.

These morphological states are not unique within the family. The bifurcation of the supratemporal is observed in *Lyopsetta exilis* on both ocular and blind side, but not all species of *Cleisthenes* and *Hippoglossoides* have the supratemporal bifurcation on the blind side. The absence of nasal bones on the blind side and number of infraorbitals are shared with some species in the Pleuronectinae, the latter being also observed in *Hippoglossus stenolepis*.

**Genus *Cleisthenes*** This genus contains two species, *C. herzensteini* and *C. pinetorum*, and is diagnosed by four synapomorphies (Fig. 14): migrated eye is near dorsal midline (25); gill rakers on fourth epibranchial present (40); a double crest or groove present on supraoccipital and blind-side frontal (38, Fig. 10, C–E); and crest extending from supraoccipital to blind side reduced (52, Fig. 10, D and E).

These morphological characters are distributed within other pleuronectid taxa but are not observed in any other Hippoglossoidinae. The position of the migrated eye is also observed in *Reinhardtius*. Presence of gill rakers on the fourth epibranchial has a limited distribution in *Eopsetta grigorjewi, E. jordani*, and *Psettichthys melanostictus*. The double crest on the supraoccipital and blind-side frontal is recurrent throughout the family but unique within Hippoglossoidinae. The reduced crest on the blind-side frontal is also observed in *Reinhardtius stomias, Pleuronichthys verticalis*, and Pleuronectini.

**Genus *Hippoglossoides*** This genus contains 4 species: *H. dubius* (not examined), *H. elassodon, H. platessoides*, and *H. robustus*, defined by two synapomorphies (Fig. 14). The structure of the anterior margin of the mesethmoid is complex but consistent in *Hippoglossoides*. In this genus, the "thin plate" structure of the mesethmoid (53, Fig. 8B) is distinct in relation to other members of Hippoglossoidinae who have a thickened triangular-shaped mesethmoid (Fig. 8C), or the plesiomorphic structure of an open canal found in *Cleisthenes pinetorum* (Fig. 8A). In addition, there are 12 to 14 abdominal vertebrae (23), an increase from 11 or fewer in other taxa. The interrelationships within *Hippoglossoides* are not fully resolved by this analysis. *Hippoglossoides dubius* has 13 abdominal vertebrae (Norman, 1934; Sakamoto, 1984a) and is assumed to have common ancestry with other species of *Hippoglossoides*.

### Subfamily Pleuronectinae

The Pleuronectinae is the largest subfamily within the Pleuronectidae with 40 species (38 examined in

this analysis). Seven synapomorphies define this group as monophyletic (Fig. 6): absence of a dentary fossa (55, Fig. 9, C and D); and absence of ceratohyal foramen (56) are both autapomorphic for Pleuronectinae; mesethmoid and blind-side prefrontal are sutured but without a foramen between these bones (14, Fig. 8D); a double supraoccipital crest forms a groove for insertion of anterior dorsal-fin pterygiophores (38, Fig. 10, C–E); a single row of teeth on lower jaw (41); intercalar in contact with basioccipital (57, Fig. 7C); and presence of a posterior extension of supratemporal branch of the lateral line (58).

Some of these character states are not found in all pleuronectines, but the occurrence of these states at basal lineages and their predominance within the Pleuronectinae indicates that the absence of these synapomorphies within the subfamily are instances of evolutionary reversal. For example, the presence of a supratemporal branch (58) is observed in 11 pleuronectine taxa and is hypothesized to arise at this node with two secondary losses observed in more advanced lineages within Microstomini and Pleuronectini.

**Intrarelationships of Pleuronectinae** The Pleuronectinae is classified into 4 tribes: Psettichthyini, Isopsettini, Microstomini, and Pleuronectini (Fig. 6). Branchial structure and characters associated with jaw asymmetry determine the interrelationships of these tribes.

**Tribe Psettichthyini Genus *Psettichthys*** Monotypic with only *P. melanostictus*, this lineage is unique within the Pleuronectinae, having six distinct characters (Fig. 6): dorsal fin rays are elongated beyond dorsal-fin membrane, an autapomorphy for the species (59); second and third basibranchials are sutured (5, Fig. 3A), a reversal within Pleuronectidae; teeth are not uniform in length (16); greater than seven infraorbital bones (51); gill rakers on fourth epibranchial present (40); and one row of teeth on upper jaw (60).

These morphological states are shared with taxa both within and prior to the Pleuronectinae. This evidence clearly positions Psettichthyini as a basal tribe of the Pleuronectinae. A suture between the second and third basibranchials is observed elsewhere only in *Limanda punctatissima*. Tooth length is uniform in other pleuronectine taxa. An increase in infraorbital number is observed in two other pleuronectine species, *Lepidopsetta bilineata* and *Pleuronichthys decurrens*, as well as in *Cleisthenes, Hippoglossoides*, and *Hippoglossus stenolepis*. The presence of gill rakers on the fourth epibranchial is found elsewhere only in *Cleisthenes* and *Eopsetta*. A single row of teeth on the upper jaw is observed in all species of the Pleuronectini, and in *Glyptocephalus* and *Microstomus*.

The second lineage contains the newly defined tribes Isopsettini, Microstomini, and Pleuronectini. Taxa within this lineage are characterized by 11 synapomorphies (Fig. 6): one gill raker at proximal base of second and third epibranchials (30, 31, Fig. 3D); blind-side premaxilla protruding past the sagittal axis at its symphysis with that of ocular side (61, Fig. 9, C and D); ocular-side premaxilla much longer than that of blind side (62, Fig. 9, C and D); ventral posterior curvature on blind-side premaxilla is present (63, Fig. 9, C and D); asymmetry in space between dentary and articular such that blind-side space is larger than on ocular side (64, Fig. 9, C and D); dorsoposterior process of ocular-side dentary larger than its blind-side counterpart (65, Fig. 9C); teeth on both ocular-side premaxilla and dentary reduced (66, 67, Fig. 9, C and D); epiotic processes present (68, Fig. 10, D and E); and ocular-side entopterygoid larger than that of blind side (69, Fig. 2, C and D).

Distribution of these character states is not without exceptions or homoplasies. Reduction of gill rakers on the second epibranchial was not observed in *Limanda* (Fig. 3C), and a reduction of gill rakers on the third epibranchial was not observed for *Pleuronectes quadrituberculatus*. These reductions are homoplastic in *Hippoglossus* and *Verasper* (Hippoglossinae).

The third lineage indicating a sister relationship between the tribes Microstomini and Pleuronectini, is based on six synapomorphies (Fig. 6): within this lineage there is an evolution of dentition, from pointed or bluntly conical teeth to incisorlike or even molariform teeth with uniform cutting edges (70); sphenotic process positioned high on sphenotic (37, Fig. 7, B and C); urohyal with strongly bifurcate cardiac apophysis (39, Fig. 12, D–F); blind-side pterosphenoid and prootic form dorsal margin of anterior prootic foramen (47, Fig. 7, B and C); medial margin of fifth ceratobranchial slightly curved (71, Fig. 3C); and teeth on fifth ceratobranchial bluntly pointed (72, Fig. 3C).

Exceptions to the distribution of character states within the third lineage are observed in few species and appear to be cases of reversal. They do not contradict the sister relationship between Microstomini and Pleuronectini. The sphenotic process is not positioned high on the sphenotic in *Glyptocephalus*. A strongly bifurcated cardiac apophysis on the urohyal was not found in *Limanda punctatissima, Pleuronichthys ritteri, P. ocellatus*, and *Parophrys vetula*. These two morphological characters are homoplastic in *Verasper* (Hippoglossinae). The sphenotic forms the dorsal margin of the anterior prootic foramen on the blind side (47, Fig. 7A) in *Limanda ferruginea, L. proboscidea, Microstomus achne, M. kitt, Glypto-*

*cephalus cynoglossus, G. stelleri, G. zachirus*, and *Pleuronichthys guttulatus*. However, the blind-side pterosphenoid and prootic uniting to form the dorsal margin of the anterior prootic foramen is homoplastic in Hippoglossoidinae and *Verasper variegatus* (Hippoglossinae). The medial curvature of the fifth ceratobranchial is not observed in *Microstomus, Glyptocephalus*, and *Pleuronichthys,* and teeth on the fifth ceratobranchial are sharply pointed in *Microstomus bathybius, Pleuronichthys*, and *Lepidopsetta*.

**Tribe Isopsettini Genus *Isopsetta*** The tribe Isopsettini is monotypic with *Isopsetta isolepis*. Four character transformations identify the lineage (Fig. 6): blind-side metapterygoid is not articulated with entopterygoid (34, Fig. 2A); anterior margin of mesethmoid forming an open canal (53, Fig. 8A); haemal spines of anteriormost caudal vertebrae broadly attached to centrum (73, Fig. 5B); and epiotics are sutured along dorsal posterior margin of skull (74).

Three of these four character states are reversals within the Pleuronectinae. The absence of an articulation between the blind-side metapterygoid and entopterygoid is a reversal of the structure observed in the Psettichthyini and in most species of Microstomini and Pleuronectini, except *Lepidopsetta, Pleuronichthys guttulatus, Limanda aspera*, and *L. ferruginea*. The open canal on the anterior margin of the mesethmoid is a reversal that is also observed in the pleuronectines, *Glyptocephalus, Microstomus*, and *Pleuronichthys* (except *P. verticalis*), and in *Cleisthenes pinetorum* (Hippoglossoidinae). Epiotics sutured along the dorsal posterior margin are observed only in one other pleuronectid species, *Microstomus pacificus*, and the broad attachment of haemal spines to the anteriormost caudal vertebrae is a reversal recurrent throughout the family, indicating a homoplastic structure with a complex distribution. Despite the reversals noted for this lineage, placement of Isopsettini within Pleuronectinae is supported by the eight synapomorphies for Pleuronectinae and the 11 derived morphological characters for the second lineage in Pleuronectinae.

**Tribe Microstomini** The tribe Microstomini contains 19 species (17 examined) classified in five genera: *Lepidopsetta, Dexistes, Pleuronichthys, Glyptocephalus*, and *Microstomus* (Fig. 15). Although the placement of this tribe within the Pleuronectinae is supported by 25 character transformations presented for monophyly and intrarelationships of the subfamily, the status of this tribe, as well as its intrarelationships, are determined mostly by character reversals. The monophyly of this tribe is characterized by four character transformations. All are reversals within the Pleuronectinae (Fig. 6): suture between mesethmoid and blind-side prefrontal is either incomplete or complete with a small foramen present (14, Fig. 8, A–C); single crest on supraoccipital (38, Fig. 10, A and B); lower jaw with multiple rows of teeth (41); and reduced or absent process on dorsoposterior edge of epiotics (68, Fig. 10, A–C).

The few exceptions to the distribution of these states within Microstomini and the occurrence of these same reversals outside of Microstomini indicate the homoplastic nature of these structures. Multiple rows of teeth on the lower jaw were not observed in *Microstomus* and *Glyptocephalus,* which is homoplastic within Hippoglossinae and recurrent for basal lineages within the family but which has only this one instance of reversal in Pleuronectinae. The absence or reduction of an epiotic crest is a reversal also found in *Pseudopleuronectes herzensteini* and *P. yokohamae*.

**Intrarelationships of Microstomini** Three lineages of Microstomini are defined by 16 character transformations; ten are reversals (Fig. 15).

**Genus *Lepidopsetta*** The first lineage of Microstomini, consists of *Lepidopsetta* containing two species, *L. bilineata* and *L. mochigarei* (Fig. 15). This genus is diagnosed by the presence of demersal eggs (75), a feature observed in only four other pleuronectid species: *Pseudopleuronectes americanus, P. schrenki, P. yokohamae*, and *P. obscurus* (not examined).

The second lineage indicates common descent for *Dexistes, Pleuronichthys, Microstomus*, and *Glyptocephalus*. Four character states unite these genera (Fig. 15): first epibranchial bifurcated at distal end (12, Fig. 3A); cardiac apophysis simple at tip with a bifurcation positioned anteriorly (39, Fig. 12, E and F), except in *Pleuronichthys ocellatus* and *P. ritteri;* intercalar not in contact with basioccipital (57, Fig. 7B), a reversal in Pleuronectinae; and less than seven teeth on ocular-side premaxilla (66).

Exceptions to character states within the second lineage of Microstomini were not congruent and did not provide alternative topologies that either excluded taxa observed to be exceptions to this distribution or included taxa that were homoplastic with these structures. Bifurcation of the first epibranchial is a reversal in the Pleuronectidae. Shape of the cardiac apophysis of the urohyal was observed only in one other pleuronectid, *Limanda aspera*. The intercalar is in contact with the basioccipital in *Glyptocephalus cynoglossus* (57, Fig. 7C). The reduction of teeth on the ocular-side premaxilla (less than 7) is not observed in *Pleuronichthys guttulatus* and *Glyptocephalus.* In *Glyptocephalus*, variation in tooth number on the ocular-side premaxilla ranged from 8 to 16 in *G. zachirus*, but in all species there is a

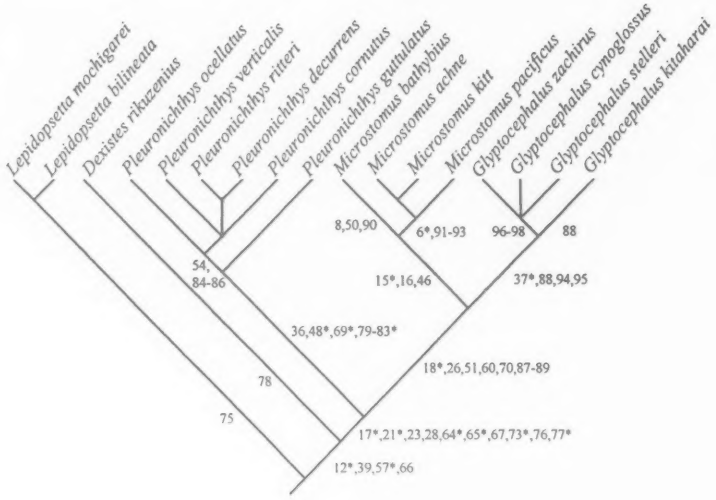


**Figure 15**

Intrarelationships of Microstomini. Numbers correspond to characters in text. Asterisk represents character state reversal.

smaller number of teeth on the ocular-side premaxilla than the number observed in previous lineages of Pleuronectinae (Norman, 1934). *Glyptocephalus cynoglossus* is reported to have 8 to 15 teeth on the ocular-side premaxilla and 17 to 26 on the associated blind-side premaxilla. *G. stelleri* has only seven teeth on the ocular-side premaxilla and 20 to 27 teeth on the blind side. *G. tanakius* has 12 to 14 teeth on the ocular-side premaxilla and 14 to 16 on the blind side, and *G. zachirus* has 12 to 16 teeth on the ocular-side premaxilla and 20 to 27 teeth on the blind side (Norman, 1934).

The third lineage of Microstomini indicates monophyly for *Pleuronichthys, Microstomus*, and *Glyptocephalus*. It is based on ten character transformations, four synapomorphies, and six reversals (Fig. 15). Synapomorphies include increased number of abdominal vertebrae to between 12 and 14 (23); lips thickened or fleshy (76); palatine of ocular side reduced and not attached to pterygoid (28, Fig. 2C); and teeth on ocular-side dentary reduced to fewer than six (67). The reversals are exoccipital and prootic in contact with each other (77, Fig. 7B); presence of an interorbital process (17, Fig. 10C); one row of gill rakers on fourth ceratobranchial (21, Fig. 3, A and B); haemal spine broadly attached to centrum (73, Fig. 5B); space between both ocular- and blind-side dentary and articular equal in size (64, Fig. 9D); and dorsoposterior process of similar size on ocular- and blind-side dentaries (65, Fig. 9D).

Exceptions and homoplasy in this distribution do not support an alternative hypothesis. *Glyptocephalus kitaharai* has only 11 abdominal vertebrae (Sakamoto, 1984a). Reduction of the ocular-side palatine was also observed in *Hippoglossus* and *Verasper* (Hippoglossinae). The exoccipital and prootic are not joined, and secondary reduction of teeth on the dentary of the ocular side are not observed in *Glyptocephalus*. These species all have proportionally fewer teeth on the ocular-side dentary, but the exact number ranges from 11 to 15 in *Glyptocephalus kitaharai* to 10 to 18 in *G. zachirus* (Norman, 1934).

Reversals in this lineage reproduce plesiomorphic states observed prior to the fourth lineage of Pleuronectidae or states that are plesiomorphic within the Pleuronectinae. The contact between exoccipital and prootic, may be a reversal for the family. The lost interorbital process in the fourth pleuronectid lineage is observed (at least partially) in these two pleuronectine genera. The single row of gill rakers on the fourth ceratobranchial is a reversal of the two rows that define the fourth pleuronectid lineage. The broad attachment of the haemal spine to the centrum is a reversal of a narrower attachment defining the fourth pleuronectid lineage. Sym-

metry in the space between dentary and articular, as well as the dentary process, are reversals for Pleuronectinae. These reversals are also observed in some species of the Pleuronectini (Table 1).

The fourth lineage in Microstomini represents the sister relationship between *Microstomus* and *Glyptocephalus*. Eight synapomorphies support this hypothesis (Fig. 15): intestine extending posteriorly into body cavity (87), unique for this lineage; more than 20 caudal-fin rays, an increase from 18 or 19 (88); more than six branched caudal-fin rays (89); 36 to 41 caudal vertebrae, an increase from 25 to 35 (26); less than five infraorbital bones, a reduction from between five and seven (51); teeth incisorlike (70); single row of teeth on upper jaw (60); and hyomandibula without a broad anteroventral margin (18, Fig. 2C).

Exceptions to the distribution of these synapomorphies do not indicate exclusion of any species defined in *Glyptocephalus* or *Microstomus*, nor does homoplasy suggest the inclusion of additional taxa. An increase in caudal-fin rays is only observed in one other pleuronectid, *Pleuronectes platessus*, which usually has 20 caudal-fin rays. An increase in the relative number of branched caudal-fin rays is also observed in *Pleuronichthys decurrens*, *P. ritteri*, and *Pseudopleuronectes yokohamae*. An increase in the number of caudal vertebrae is homoplastic in *Reinhardtius* (Hippoglossinae). A reduction in the number of infraorbital bones is not observed in *Glyptocephalus kitaharai* and *Microstomus pacificus* but is reduced in *Pseudopleuronectes americanus*. The incisorlike tooth structure is unique within Microstomini but is observed in *Parophrys vetula*, *Pleuronectes*, and *Pseudopleuronectes*. A single row of teeth on the upper jaw is also unique within Microstomini but is also observed in *Psettichthys melanostictus* (Psettichthyini) and Pleuronectini. The absence of a broadened hyomandibula is a reversal unique within this lineage of *Glyptocephalus* and *Microstomus*. All other taxa within the fourth lineage of Pleuronectidae have a hyomandibular with a broadened anterior margin (Fig. 2D).

**Genus *Dexistes*** The monotypic genus contains *D. rikuzenius* (Fig. 15). The monotypic status of this species is based on the morphological characters examined for intrarelationships of Microstomini and the presence of an ocular-side postocular ridge (78). The latter character has an additional evolutionary step within the sister tribe Pleuronectini, uniting a clade comprising *Limanda*, *Platichthys*, *Pleuronectes*, and *Pseudopleuronectes*.

**Genus *Pleuronichthys*** This genus contains seven species: *Pleuronichthys coenosus* (not examined), *P. cornutus*, *P. decurrens*, *P. guttulatus*, *P. ritteri*, *P. ocellatus*, and *P. verticalis*. This genus is identified by eight character transformations (Fig. 15): presence of villiform teeth, autapomorphic for *Pleuronichthys* (79); large foramen in blind-side prefrontal (80); gill rakers on second hypobranchial reduced to one at proximal base (36); lateral process present on ocular-side frontal (81, Fig. 7B, 10C); 25 or less caudal vertebrae (82); ocular-side pterosphenoid reduced and not forming posterior margin of orbit (83, Fig. 7B); blind-side pterosphenoid is similarily reduced (48, Fig. 7B); and ocular-side entopterygoid similar in size to that of blind side (69, Fig. 2, A and B). *Pleuronichthys coenosus* is assumed to be a member of this genus on the basis of presence of villiform teeth, reduced number of caudal vertebrae (24 to 25), and presence of a lateral process on ocular-side frontal (Sakamoto, 1984a).

There are few exceptions to the distribution of character states within *Pleuronichthys*, and instances of homoplasy do not indicate an alternative hypothesis. The large foramen in the blind-side prefrontal was observed in only one other species, *Glyptocephalus stelleri*. Reduction of gill rakers on the second hypobranchial was not observed in *Pleuronichthys ocellatus*, which has at least two gill rakers on the second hypobranchial. The presence of a lateral process on the ocular-side frontal is also observed in *Microstomus achne*, *M. kitt*, and *M. pacificus*. However, it is not nearly as distinct as that in *Pleuronichthys*. Only *Platichthys flesus*, *P. stellatus*, and *Pleuronectes putnami* (Pleuronectini) have also fewer than 25 caudal vertebrae. Reduction of the ocular-side pterosphenoid is not observed in *Pleuronichthys ritteri*, where the pterosphenoid separates frontal and parasphenoid to form the posterior margin of the orbit. Reduction of the blind-side pterosphenoid is also observed in *Acanthopsetta* and *Hippoglossoides* (Hippoglossoidinae). These two character states are reversals in the Pleuronectidae. The symmetry between ocular-side and blind-side entopterygoids is a reversal of the asymmetrical structure observed prior to the Isopsettini. This reversal only occurs in *Pleuronichthys* and *Glyptocephalus kitaharai*.

The intrarelationships of *Pleuronichthys* are not fully resolved. *Pleuronichthys guttulatus* is the sister species to all other species of *Pleuronichthys* (Fig. 15). Four synapomorphies unite *P. cornutus*, *P. coenosus*, *P. decurrens*, *P. ocellatus*, *P. ritteri*, and *P. verticalis*: dorsal fin originates on blind side of head (84); reduced or absent cartilaginous interspace between blind-side prefrontal and parasphenoid (85, Fig. 7B); mesethmoid forms only part of anterior margin of upper orbit (86); and ocular-side metapterygoid articulated with entopterygoid (54). This last feature is homoplastic because it is also observed in *Reinhardtius hippoglossoides* and *Limanda punctatissima*.

**Genus *Microstomus*** This genus contains five species: *M. achne, M. bathybius, M kitt., M. pacificus*, and *M. shuntovi* (not examined) and is defined by three synapomorphies (Fig. 15): posterior extension of supraoccipital absent (46, Fig. 10B); first anal pterygiophore thin (15); teeth in both upper and lower jaws uniform in length forming a continuous cutting edge (16, Fig. 9D). Descriptions of *Microstomus shuntovi* indicate that this species is very similar to *M. kitt* (Borets, 1983). The teeth are described as "chisel-like" (Borets, 1983), a term that similarly describes teeth in other species of *Microstomus*. An examination of the supraoccipital and the first anal pterygiophore would verify this classification.

Absence of the posterior extension on the supraoccipital has evolved independently in the Hippoglossoidinae. The thin structure of the first anal pterygiophore is a reversal of a thickened structure defining the third lineage. The continuous cutting edge of the teeth is an advanced state, also present in *Glyptocephalus zachirus, Pleuronectes glacialis, P. pinnifasciatus, P. putnami*, and *Pseudopleuronectes schrenki*.

*Microstomus bathybius* is the sister species to *M. achne, M. kitt*, and *M. pacificus* (Fig. 15). This deep-sea species is unique in being the only pleuronectid with a series of infraorbital bones on the ocular side (8). It is the only species of *Microstomus* in which the blind-side nasal bone is absent (50) and is the only pleuronectid in which the anteroventral tip of the pelvis is anterior to the cleithrum (90). The other species of *Microstomus* share four character states (Fig. 15): ocular-side scales with radii completely surrounding the focus (91); margins of interoperculum and suboperculum fimbriated (92, 93, Fig. 2C); and haemapophysis present on most posterior abdominal vertebrae (6, Fig. 4D). This last feature is a reversal for Pleuronectidae, and only *Glyptocephalus zachirus* has also a fimbriated subopercular margin.

**Genus *Glyptocephalus*** This genus contains four species: *G. cynoglossus, G. kitaharai, G. stelleri*, and *G. zachirus*. Four synapomorphies are hypothesized at this node (Fig. 15): more than 21 caudal-fin rays (88); cleithra inserted by tip of urohyal (94); presence of two to four pyloric appendages and two or three on upper intestine, an increase from two to three and one on upper intestine (95); sphenotic process positioned low on sphenotic and forming dorsal roof of hyomandibular socket (37, Fig. 7A). This last character is a reversal of that observed in all other Microstomini and Pleuronectini.

*Glyptocephalus kitaharai* is the sister species to *G. cynoglossus, G. stelleri*, and *G. zachirus*. It is unique within this clade and with all other pleuronectids in having 23 caudal-fin rays, the highest number observed in the family (88). The other species are united by three character states (Fig. 15): blind-side nasal bones larger than those of ocular side (96); development of large mucous cavities on blind-side head (97), both characters unique for these three species; and presence of an interpterosphenoid bar (98, Fig. 16), not observed in any other species of *Glyptocephalus* or immediate lineages, but homoplastic in *Hippoglossoides, Acanthopsetta, Psettichthys, Limanda aspera, L. ferruginea, L. proboscidea, L. punctatissima, Pseudopleuronectes americanus, P. yokohamae*, and *Pleuronectes*. The monophyletic status of these three species is in agreement with relationships inferred through an analysis of body shape (Chiu, 1990).

**Tribe Pleuronectini** The tribe Pleuronectini contains 20 species (19 examined). Four synapomorphies define this tribe (Fig. 6): at least two regular rows of teeth on fifth ceratobranchial (49, Fig. 3, C and D); postocular ridge present on ocular side (78); upper jaw teeth in single row (60); and dorsal crest extending anteriorly from supraoccipital to blind-side frontal reduced or absent (52, Fig. 10, D and E).

Exceptions and homoplasy did not indicate an alternative hypothesis for Pleuronectini; all 19 species examined were grouped in all 128 of the equally par-

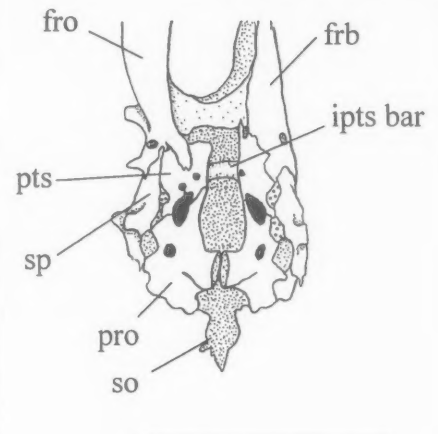


**Figure 16**

Ventral aspect of cranium, *Pleuronectes quadrituberculatus* USNM 064042, with parasphenoid removed to reveal interpteroshenoid bar. frb= frontal (blind side); fro, frontal (ocular side); ipts bar = interpterosphenoid bar; pro = prootic; pts = pteroshenoid; so = supraoccipital; sp = sphenotic. Anterior region of orbit and occipital region have been removed. Scale bar is 10 mm.

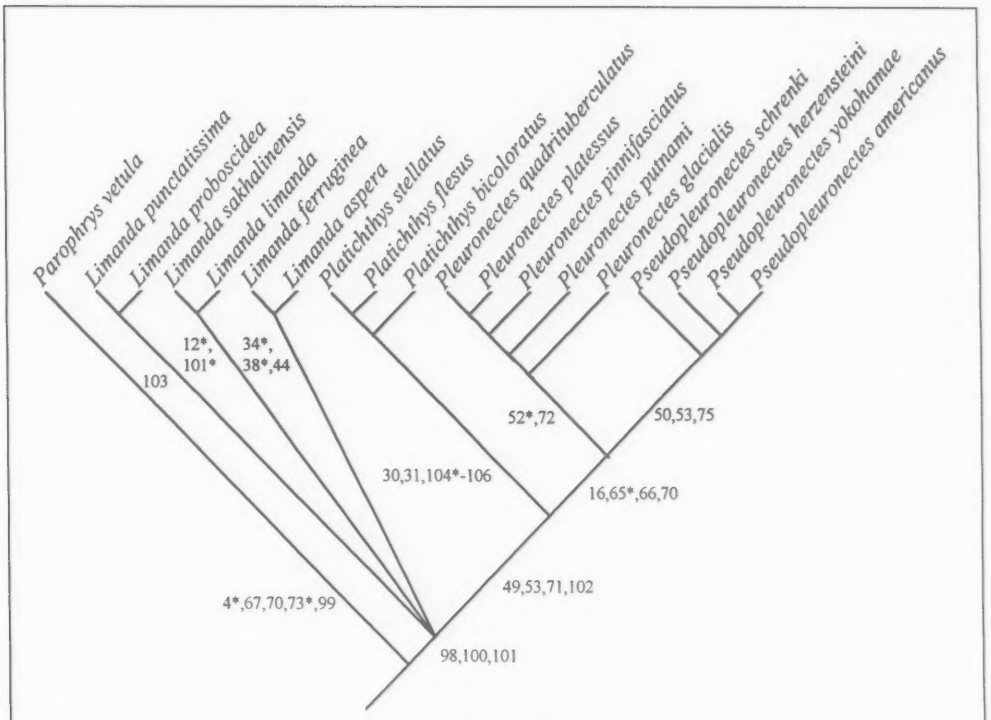


**Figure 17**

Intrarelationships of Pleuronectini. Numbers correspond to characters in text. Asterisk represents character state reversal.

simonious trees. A postocular ridge is observed in only one other pleuronectid taxon, *Dexistes rikuzenius*. A single row of teeth in the upper jaw was also observed in *Psettichthys melanostictus*, *Glyptocephalus*, and *Microstomus*. The structure of the dorsal crest, extending along the supraoccipital and blind-side frontal, was homoplastic within Pleuronectini and in other pleuronectid lineages. *Limanda aspera, Pleuronectes quadrituberculatus, P. glacialis, P. pinnifasciatus*, and *P. putnami* retain a prominent crest. *Pleuronichthys verticalis, Cleisthenes*, and *Reinhardtius stomias* share the reduced morphological character with Pleuronectini.

### Intrarelationships of Pleuronectini

**Genus *Parophrys*** The first lineage in Pleuronectini has only one species, *Parophrys vetula*, which is distinct with five character transformations (Fig. 17): suture between first and second basibranchial (4, Fig. 3A); haemal spines broadly attached to ventral surface of centrum (73), are both reversals within Pleuronectidae; absence of dentary foramen on ocular side (99); reduction of teeth on ocular-side dentary (67) and incisorlike teeth (70, Fig. 9D) are shared with higher lineages within Pleuronectini but are homoplastic, and observed in Microstomini and species of Hippoglossinae.

*Limanda, Platichthys, Pleuronectes*, and *Pseudopleuronectes* represent the second lineage in Pleuronectini and are hypothesized to share an immediate common ancestor on the basis of three synapomorphies (Fig. 17): presence of a blind-side postocular ridge (100); presence of bony prominences on ocular-side postocular ridge (101, Fig. 10E); and presence of an interpterosphenoid bar (98, Fig. 16). The blind-side postocular ridge and bony prominences on the ocular-side postocular ridge are unique within Pleuronectidae. The latter was not observed in *Limanda limanda, L. sakhalinensis, L. aspera, Pseudopleuronectes herzensteini, P. yokohamae*, and *P. americanus*. The presence of an interpterosphenoid bar was not observed in *Limanda limanda, L. sakhalinensis, Pseudopleuronectes schrenki*, and *P. herzensteini* and is homoplastic in *Glyptocephalus cynoglossus, G. stelleri, G. zachirus, Psettichthys, Acanthopsetta*, and *Hippoglossoides*.

The third lineage of Pleuronectini is a clade uniting *Platichthys, Pleuronectes*, and *Pseudopleuronectes*. This result was observed in 80 of 128 (62.5%) trees found by heuristic search and is supported by four morphologies (Fig. 17): bony prominences on blind-side postocular ridge (102, Fig. 10E); a strong medial curvature on fifth ceratobranchial resulting in close approximation or union of ceratobranchials (71, Fig. 3D); an increase of rows of teeth on fifth ceratobranchial from two regular rows to multiple rows of regular length (49, Fig. 3D); and anterior margin of mesethmoid (53) either a thin plate (Fig. 8B) or thickened triangular edge (Fig. 8C).

Exceptions and homoplasy observed in these morphological characters contributed to three alternative topologies for intrarelationships of Pleuronectini (Fig. 18, A–C). Bony prominences on the blind-side postocular ridge were not observed in *Pseudopleuronectes*, *Pleuronectes platessus*, and *P. quadrituberculatus* and are homoplastic in *Limanda proboscidea* and *L. punctatissima*. Both attributes of the fifth ceratobranchial, the close approximation of the medial margins and the multiple rows of teeth, are not observed in *Pseudopleuronectes*. The thin plate structure of the mesethmoid (Fig. 8B), observed in *Pseudopleuronectes* and *Pleuronectes platessus* is homoplastic in *Dexistes rikuzenius*, *Pleuronichthys verticalis* (Microstomini), and *Hippoglossoides* (Hippoglossoidinae). The thickened triangular edge of the mesethmoid (Fig. 8C) is unique within

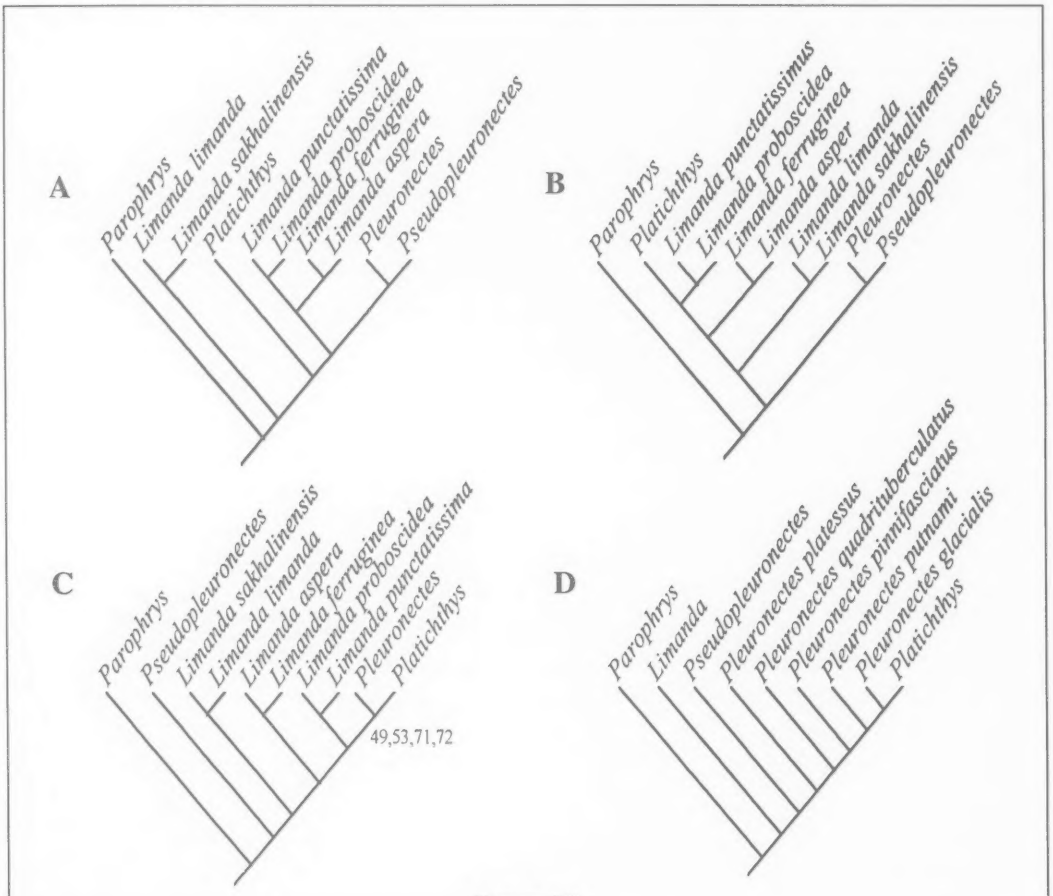


**Figure 18**

Alternative topologies for intrarelationships of Pleuronectini. (**A, B, and C**) Alternative topologies observed for the six species in *Limanda*. Each topology was resolved 16 of the 128 equally parsimonious cladograms. (**D**) Alternative topology for interrelationships in *Pleuronectes* observed in 16 of 128 equally parsimonious cladograms. Numbers correspond to characters in text.

Pleuronectidae but is not observed in *Pseudopleuronectes*, *Pleuronectes platessus*, and *P. quadrituberculatus*. The three alternatives are equally represented in 48 (37.5%) of the most parsimonious results but are not illustrated in the 50% majority-rule consensus tree (Fig. 1). All three alternatives (Fig. 18) maintain monophyly for *Platichthys*, *Pleuronectes*, and *Pseudopleuronectes*, individually. These alternatives place some or all species of *Limanda* as paraphyletic within a *Platichthys*, *Pleuronectes*, *Pseudopleuronectes* clade but do not exclude any species of *Platichthys*, *Pleuronectes*, and *Pseudopleuronectes*. Character states in support of these conflicting nodes illustrate homoplasy observed in *Limanda* (Fig. 18, A–C). Some or all species of *Limanda* are observed to share these character states with the other three genera. However, most of the exceptions and homoplasy observed in these structures fail to illustrate an evolutionary hypothesis that is more convincingly corroborated than the topology presented in the consensus tree (Fig. 1).

The fourth lineage unites *Pleuronectes* and *Pseudopleuronectes*. This clade was observed in 75% of the equally parsimonious trees (Fig. 1) and is supported by four synapomorphies (Fig. 17): less than six teeth on ocular-side maxilla (66, Fig. 9D); teeth incisorlike (70, Fig. 9D); teeth forming a continuous cutting edge (16, Fig. 9D); and dorsoposterior process of dentary similar in size on both ocular and blind sides (65, Fig. 9D), a reversal of the second lineage of Pleuronectinae.

Homoplasy observed in these morphological characters reveals a pattern of parallel evolution between this lineage and the clade formed by *Glyptocephalus*, *Microstomus*, and *Pleuronichthys* (Microstomini). Less than six teeth on the ocular-side premaxilla are also present in these three genera of Microstomini. The presence of incisorlike teeth is also observed in *Parophrys vetula* as well as *Glyptocephalus* and *Microstomus*. A continuous cutting edge found on the teeth has independently evolved in *Glyptocephalus* and *Microstomus*. The symmetrically sized dorsoposterior process of the dentary is not observed in *Pseudopleuronectes herzensteini*, *Pleuronectes platessus*, and *P. quadrituberculatus* and is a reversal that is paralleled in *Glyptocephalus* and *Microstomus*.

Alternative topologies observed in 32 of 128 (25%) trees unite either a monophyletic or paraphyletic *Pleuronectes* with *Platichthys* (Fig. 18, C and D). In *Pleuronectes* and *Platichthys* the mesethmoid has a thickened, triangular anterior margin (53, Fig. 8C). The fifth ceratobranchial has a strong medial curve forming a triangular plate (71, Fig. 3D). The teeth of the fifth ceratobranchial are in multiple rows (49, Fig. 3D), and these teeth are generally rounded or molariform (72, Fig. 3D). Three of these characters (49, 53, 71) are used to define the third lineage of Pleuronectini but notably exclude *Pseudopleuronectes*. The molariform teeth on the fifth ceratobranchial (72, Fig. 3D) are observed in *Pleuronectes* and only in *Platichthys bicoloratus*. The alternative hypothesis, indicating a sister relationship between *Pleuronectes* and *Platichthys*, has the same character support as the hypothesis presented in the consensus tree. However, this topology is parsimonious in only 32 trees and only in conjunction with one of the two alternatives that either position a paraphyletic *Limanda* after *Pseudopleuronectes* (Fig. 18C) or hypothesize a paraphyletic *Pleuronectes* (Fig. 18D). Support for either of these topologies is not well corroborated. Therefore, alternatives to the consensus tree are not as robust to the homoplasy observed in the analysis.

**Genus *Limanda*** Monophyly and intrarelationships of *Limanda* are unresolved by this analysis owing to homoplasy observed in these six species (Fig. 17). *Limanda punctatissima* and *L. proboscidea* are the only two pleuronectid species with bony prominences of the postocular ridge extending anteriorly onto the interorbital bar (103, Fig. 10E). *Limanda aspera* and *L. ferruginea* are united by three morphological characters: the supraoccipital crest not forming a groove for the dorsal-fin pterygiophores (38, Fig. 10, A and B); blind-side metapterygoid not articulated with entopterygoid (34, Fig. 2A), both reversals in Pleuronectini; and supratemporal of the blind side jointed at its anterior bifurcation (44, Fig. 13B). This character state has evolved independently five times in Pleuronectidae as it is also observed in *Cleisthenes herzensteini*, *Hippoglossoides elassodon*, *Lyopsetta exilis*, and *Reinhardtius hippoglossoides*. *Limanda limanda* and *L. sakhalinensis* are monophyletic in 75% of the trees (Fig. 1). Two character states unite these species: first epibranchial is bifurcated (12, Fig. 3A); and bony prominences on ocular-side postocular ridge absent (101). Both of these characters are reversals also observed in *Limanda aspera*. Alternative topologies observed in 32 (25%) other trees, place *Limanda sakhalinensis* and *L. limanda* as paraphyletic taxa in the tribe or as paraphyletic within a clade that unites these two species with *Limanda punctatissima* and *L. proboscidea*.

Although the species of *Limanda* are not resolved as a monophyletic group, they are distinguishable from other members of Pleuronectini. They have reduced dentition on the ocular side but maintain more than six teeth, where as other species of Pleuronectini generally have six or fewer (Norman, 1934). The teeth are bluntly conical or pointed with truncated tips, whereas all other members of this tribe have com-

pressed teeth with an incisorlike shape (Norman, 1934; Sakamoto, 1984a). Both of these tooth structures in *Limanda* are interpreted as synapomorphies for the Pleuronectini. There is little evidence in support for the monophyly of *Limanda*. Only one character state is shared by all six species: presence of gill rakers on second epibranchial (30, Fig. 3C). This character is a reversal of a reduced number of gill rakers defining the second lineage of Pleuronectinae (Fig. 6). There were 16 trees of 403 steps that hypothesized a monophyletic *Limanda* based on three reversals (Fig. 18D), including the presence of gills rakers on the second epibranchial. All 16 topologies were correlated with a hypothetical alternative to the consensus tree (Fig. 18D) such that a monophyletic hypothesis for *Limanda* is not resolved by consensus and is therefore not robust to the homoplasy observed in the analysis. However, the placement of these six species near the base of the second lineage in Pleuronectini and the shared morphological characters mentioned above suggest that a conservative approach to their nomenclature should be considered and that these six species should remain classified as *Limanda* until a more focused phylogenetic analysis is performed.

**Genus *Platichthys*** This genus contains three species: *P. bicoloratus, P. flesus*, and *P. stellatus*. It is monophyletic with five synapomorphies (Fig. 17): scales along median fins absent (104), a possible reversal in Pleuronectidae; scales on the body modified to form bony tubercles (105); gill rakers on second and third epibranchial absent (30, 31); and supratemporals on ocular and blind sides are fused to the cranium (106). Absence of gill rakers on the second epibranchial is observed only in the distant lineage *Verasper*. The absence of gill rakers on the third epibranchial also occurs in *Verasper* and *Pleuronectes platessus*. The ocular-side supratemporal fused to the cranium is homoplastic in *Limanda punctatissima*.

**Genus *Pleuronectes*** This genus contains five species: *P. glacialis, P. pinnifasciatus, P. platessus, P. putnami*, and *P. quadrituberculatus*. The monophyly of these five species was observed in 112 of 128 trees (87.5%) and is supported by two synapomorphies (Fig. 17): prominent dorsal crest extending from the supraoccipital to the blind-side frontal (52, Fig. 10, B and C); and presence of molariform teeth on fifth ceratobranchial (72, Fig. 3D). A prominent crest extending from the supraoccipital to blind-side frontal is a reversal within Pleuronectini, that is also observed in *Limanda aspera*. The presence of molariform teeth on the fifth ceratobranchial is only shared with *Platichthys bicoloratus*.

Alternative topologies observed in 16 of 128 trees (12.5%) place species of *Pleuronectes* as paraphyletic with *Platichthys stellatus, P. flesus*, and *P. bicoloratus* at the terminal end (Fig. 18D). This topology does not contradict the synonymy of *Liopsetta* (*sensu* Norman) within *Pleuronectes*. It does contradict the classification of *Platichthys*. However, as previously illustrated, the alternative topologies for the intrarelationships within Pleuronectini are based on homoplasy observed in *Limanda* such that the paraphyletic origin in *Pleuronectes* is correlated and observed in the same 16 trees indicating monophyly of *Limanda* (Fig. 18D).

**Genus *Pseudopleuronectes*** This genus contains five species: *P. americanus, P. herzensteini, P. obscurus* (not examined), *P. yokohamae*, and *P. schrenki*. The monophyletic status of this group is supported by three synapomorphies (Fig. 17): anterior margin of mesethmoid forming a thin plate (53, Fig. 8B); blind-side nasal bone absent (50); and demersal eggs (75). *Pseudopleuronectes obscurus* has character states synapomorphic for the fourth lineage of Pleuronectinae, uniting *Pleuronectes* and *Pseudopleuronectes*. These are the presence of incisorlike teeth forming a continuous cutting edge and close approximation of the fifth ceratobranchials (Norman, 1934). Although teeth on the fifth ceratobranchial are bluntly conical (Norman, 1934), as in *Pseudopleuronectes*, this character state is plesiomorphic at this phylogenetic level. However, presence of a demersal egg in *P. obscurus* (Hensley and Ahlstrom, 1984), is synapomorphic for *Pseudopleuronectes*.

Homoplasy and two exceptions observed in these morphological characters do not corroborate an alternative hypothesis. A clade uniting the four species of *Pseudopleuronectes* examined here, is observed in all 128 equally parsimonious cladograms. The thin plate structure of the mesethmoid is also observed in *Pleuronectes platessus*, and in more distant taxa, *Pleuronichthys verticalis*, *Dexistes rikuzenius*, and *Hippoglossoides*. The absence of a blind-side nasal bone is homoplastic in *Pleuronectes platessus* and *Limanda* (except *L. sakhalinensis*). It is also observed outside of Pleuronectini in *Microstomus bathybius*, *Pleuronichthys* (except *P. guttulatus*), *Cleisthenes*, and *Hippoglossoides*. A demersal egg is not observed in *P. herzensteini* and is homoplastic in *Lepidopsetta*.

## Summary of intrarelationships

The species examined in this analysis (53 of 59), represent the complete range of morphological variation found in the family. The character analysis outlines the characters synapomorphic for clades revealed in the consensus tree (Fig. 1).

The Pleuronectidae can be summarized as comprising large piscivorous species at the basal lineages,

whose jaws and dentition are nearly symmetrical, and species possessing more specialized dentition, jaw structure, and diverse feeding habits at subsequent lineages. The monophyly and intrarelationships of basal lineages in the Pleuronectidae are not supported by a large number of synapomorphies. This is inherent for taxa associated with basal lineages in which the majority of morphological features are plesiomorphic with respect to the ingroup under examination (Stiassny and de Pinna, 1994). However, the position of these basal lineages is key in establishing the polarity of character states for the more advanced taxa in Pleuronectidae.

Homoplasy observed in most of the 106 morphological characters was expected in an analysis containing this many taxa. The alternative topologies within Pleuronectini (Fig. 18) were supported by homoplasy but do not have better character support than the intrarelationships determined through consensus. These alternative topologies are not any less parsimonious than the topologies summarized by the consensus tree. Instead, the limited occurrence of these alternatives indicates that these topologies are not as robust to homoplasy as those summarized in the consensus tree. The taxonomic nomenclature established by the consensus tree is not in contradiction with alternatives. Based on the cladogram, this classification represents the most conservative nomenclature for such a diverse group of pleuronectids.

The interrelationships within most genera cannot be confidently supported, either because of a lack of observed morphological variation (as in *Glyptocephalus, Hippoglossoides*, and *Pleuronichthys*) or because of the large amount of homoplasy occurring at terminal nodes indicated by the low consistency index (ci=0.33) and a high retention index (ri=0.79), as in *Limanda, Pleuronectes, Pseudopleuronectes*, and *Platichthys*. In either instance, species interrelationships for these taxa do not warrant a formal description based on synapomorphies from this study. Additional morphological characters, such as the number of dorsal- and anal-fin rays, homoplastic at higher levels of universality may prove informative for species-level relationships. The developmental information summarized in Ahlstrom et al. (1984) has been expanding owing to the commercial popularity of many pleuronectid species (for example Fukuhara, 1988; Markle et. al., 1992). Yet there are still many pleuronectid species for which information of this kind is not yet available. Despite this lack of information across the family, it is certain that these developmental data can also be used in the future to help resolve natural groups among well-documented pleuronectid species.

The phylogenetic limitations of jaw and dental morphological characters is clearly illustrated in the convergent evolution of jaw structures observed between the tribes Microstomini and Pleuronectini. This is most evident in the dentition of *Glyptocephalus*, and *Microstomus*, compared with *Platichthys, Pleuronectes*, and *Pseudopleuronectes*, both of which have single row of incisorlike teeth that sometimes form a continuous cutting edge. However, monophyly for these taxa, based on jaw morphology and dentition, is not supported by other character states observed in the cranial bones and branchial apparatus. These other characters clearly resolve the monophyletic status and intrarelationships of these two groups within each of the two tribes, Microstomini and Pleuronectini. The trend of character reversal, 19 in total (Figs. 6 and 15), observed in Microstomini suggests that some members of this taxon may be positioned at a more basal node in Pleuronectidae prior to the fourth lineage. However, this alternative is not supported in any of the 128 most parsimonious trees. This analysis clearly places Microstomini as a lineage within the Pleuronectinae on the basis of 28 character states that were not reversals.

Six pleuronectid species were not examined in this analysis. They are *Clidoderma asperrimum*, *Hippoglossoides dubius*, *Microtomus shuntovi*, *Pseudopleuronectes obscurus*, *Pleuronichthys coenosus*, and *Reinhardtius evermanni*. These species are classified as members of Pleuronectidae on the basis of morphological characters obtained from the literature. In addition, their phylogenetic position is based on examination of external characters, radiographs, and literature. The *a posteriori* classification of these six species lends support for the functionality of this analysis, such that an individual species can be classified on the basis of uniquely derived features.

## Classification

The objectives for a formal classification of the Pleuronectidae were to recognize large groups within the family and to ensure that only natural groups are represented in the classification. This implied a revision of the established nomenclature. However, changes were minimal and aimed mainly at simplifying the existing nomenclature with its many monotypic genera.

Our results indicate that the Pleuronectidae is monophyletic and can be subdivided into 5 new subfamilies: Hippoglossinae, Eopsettinae, Lyopsettinae, Hippoglossoidinae, and Pleuronectinae. This classification is based on the 50% majority-rule consensus of 128 parsimony trees, obtained through heuristic search (Fig. 1). This is the first classification based on a complete phylogenetic analysis of the group. It

provides a simplified yet phylogenetically informative framework for future studies involving this family.

## Taxonomic list of the Pleuronectidae

Subfamily **Hippoglossinae**
Genus *Reinhardtius* (*R. evermanni*,[2] *R. hippoglossoides*, *R. stomias*)
Genus *Hippoglossus* (*H. hippoglossus*, *H. stenolepis*)
Genus *Verasper* (*V. moseri*, *V. variegatus*)
Genus *Clidoderma* (*C. asperrimum*[2])
Subfamily **Eopsettinae**
Genus *Eopsetta* (*E. grigorjewi*, *E. jordani*)
Subfamily **Lyopsettinae**
Genus *Lyopsetta* (*L. exilis*)
Subfamily **Hippoglossoidinae**
Genus *Acanthopsetta* (*A. nadeshnyi*)
Genus *Cleisthenes* (*C. herzensteini*, *C. pinetorum*)
Genus *Hippoglossoides* (*H. dubius*,[2] *H. elassodon*, *H. platessoides*, *H. robustus*)
Subfamily **Pleuronectinae**
Tribe **Psettichthyini**
Genus *Psettichthys* (*P. melanostictus*)
Tribe **Isopsettini**
Genus *Isopsetta* (*I. isolepis*)
Tribe **Microstomini**
Genus *Lepidopsetta* (*L. bilineata*, *L. mochigarei*)
Genus *Dexistes* (*D. rikuzenius*)
Genus *Pleuronichthys* (*P. coenosus*,[2] *P. cornutus*, *P. decurrens*, *P. guttulatus*, *P. ocellatus*, *P. ritteri*, *P. verticalis*)
Genus *Microstomus* (*M. achne*, *M. bathybius*, *M. kitt*, *M. pacificus*, *M. shuntovi*[2])
Genus *Glyptocephalus* (*G. cynoglossus*, *G. kitaharai*, *G. stelleri*, *G. zachirus*)
Tribe **Pleuronectini**
Genus *Parophrys* (*P. vetula*)
Genus *Limanda*[3] (*L. aspera*, *L. ferruginea*, *L. limanda*, *L. proboscidea*, *L. punctatissima*, *L. sakhalinensis*)
Genus *Platichthys* (*P. bicoloratus*, *P. flesus*, *P. stellatus*)
Genus *Pleuronectes* (*P. glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, *P. quadrituberculatus*)
Genus *Pseudopleuronectes* (*P. americanus*, *P. herzensteini*, *P. obscurus*,[2] *P. schrenki*, *P. yokohamae*)

[2] Species not included in analysis, phylogenetic position and classification determined *a posteriori*.
[3] Monophyletic status is uncertain.

The reclassification of several species is necessary to incorporate the new phylogenetic information. The changes to the classification are as follows:

1 *Atherestes evermanni*, *A. stomias*, and *Reinhardtius hippoglossoides* are united within *Reinhardtius* which is the oldest valid genus-group name. *Reinhardtius* (=*Atherestes*) *evermanni* was not examined and its position within the subfamily Hippoglossinae was determined *a posteriori*. Placement of *R. evermanni* within the clade uniting these three species is based on examination of external morphological characters, number of abdominal vertebrae, and the description provided in Norman (1934).
2 The genus *Eopsetta* (*sensu* Sakamoto, 1984a) with *E. exilis*, *E. grigorjewi*, and *E. jordani* is paraphyletic. The revised *Eopsetta* contains *E. grigorjewi* and *E. jordani* whereas *E. exilis*, is reassigned to the genus *Lyopsetta* as in Norman (1934).
3 *Hippoglossoides herzensteini* and *H. pinetorum* (*sensu* Sakamoto, 1984a) are reclassified as *Cleisthenes*, as in Norman (1934). This is a subjective reclassification because interrelationships of these two species within Hippoglossoidinae does not contradict the previous classification. It is based on the monophyletic status of these two species and maintains a hierarchically consistent nomenclature within the family.
4 A number of revisions were made to produce a simplified nomenclature that is hierarchically consistent with the phylogenetic interrelationships. Species of *Hypsopsetta* and *Pleuronichthys* (*sensu* Sakamoto, 1984a) are regrouped under *Pleuronichthys*. The species-group name (*guttulata*) is revised to agree with gender in the genus (i.e. *Pleuronichthys guttulatus*). The species of *Embassichthys* and *Microstomus* (*sensu* Sakamoto, 1984a) are regrouped under *Microstomus*. The species of *Errex*, *Glyptocephalus*, and *Tanakius* (*sensu* Sakamoto, 1984a) are regrouped under *Glyptocephalus*. The species of *Kareius* and *Platichthys* (*sensu* Sakamoto, 1984a) are regrouped under *Platichthys*.
5 The genus *Pleuronectes* (*sensu* Sakamoto, 1984a), with 19 species, is not monophyletic. According to our results *Pleuronectes* comprises only five species: *P. glacialis*, *P. pinnifasciatus*, *P. platessus*, *P. putnami*, and *P. quadrituberculatus*. The species-group name (*platessa*) is revised to correspond with gender in the genus (i.e. *Pleuronectes platessus*). Other species of *Pleuronectes* (*sensu* Sakamoto, 1984a) are reclassified as *Lepidopsetta bilineata*, *L. mochigarei*, *Parophrys vetula*, *Limanda aspera*, *L. ferruginea*, *L. limanda*, *L.*

*sakhalinensis*, *L. proboscidea*, *L. punctatissima*, *Pseudopleuronectes americanus*, *P. herzensteini*, *P. obscurus* (not examined), *P. schrenki*, and *P. yokohamae* on the basis of the oldest valid genus-group name for each species, with the exception of *Pseudopleuronectes schrenki*, and *P. obscurus*, previously classified as *Limanda schrenki* (Sakamoto, 1984b; Hensley and Ahlstrom, 1984) and *Liopsetta obscura* (Norman, 1934), respectively. Sakamoto's (1984a) classification was based on a 100% similarity observed among these 19 species. The new phylogeny is based on 67 additional morphological features that clearly differentiate and classify these 19 species.

6 The reclassification of *Pseudopleuronectes schrenki* is based on its position in all 128 most parsimonious trees, none of which united *P. schrenki* with other taxa exclusive of the three other species in *Pseudopleuronectes*. In addition to the three synapomorphies uniting *Pseudopleuronectes*, the most notable characteristic of *P. schrenki* in observation and in the literature is the presence of incisorlike teeth that form a continuous cutting edge (character 70, Fig. 9D, see also Sakamoto, 1984b). This character defines the fourth lineage of Pleuronectini, uniting *Pseudopleuronectes* and *Pleuronectes*, and excludes the other species of *Limanda* that have teeth with truncated tips or are bluntly conical (70, Fig. 9C). A review of literature describing *Pseudopleuronectes schrenki* (=*Limanda schrenki*) indicates a close relationship with *P. yokohamae* (=*Limanda yokohamae*) (Jordan and Starks, 1906). Norman (1934) synonymized *Limanda schrenki* under *Pseudopleuronectes yokohamae*. Studies dealing with egg characteristics also seem to synonymize *Pseudopleuronectes schrenki* with *P. yokohamae* (Yusa, 1960; Pertseva-Ostroumova, 1961). Recent studies (Sakamoto, 1984b) indicate that *P. schrenki* (=*Limanda schrenki*) is a valid species with similar characteristics to *P. yokohamae* (=*Limanda yokohamae*). Meristic counts of vertebrae, dorsal- and anal-fin rays are overlapping (Sakamoto, 1984a, 1984b). They also have similar dental structure. However, *P. schrenki* has a distinct pattern of bars on the dorsal- and anal-fin rays (Sakamoto, 1984b). Uncertainty surrounding the status of *Pseudopleuronectes schrenki* is also the result of the ineffective use of nomenclature. As illustrated in the above references, the classification of *Pseudopleuronectes yokohamae* as *Limanda yokohamae*, has led to a degree of confusion, further supporting the need to clearly identify natural groups on the basis of uniquely derived features. Although a further analysis is required to clearly assess the taxonomic status of *Pseudopleuronectes schrenki* and *P. yokohamae*, this analysis clearly indicates that neither should be classified as *Limanda*.

7 The reclassification of *Pseudopleuronectes obscurus* is based solely on information available from the literature. This species is confidently placed in the fourth lineage of Pleuronectini and its placement as a species of *Pseudopleuronectes* is supported by one synapomorphy, the presence of a demersal egg. A complete osteological examination of this species may test the validity of this classification.

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## Literature cited

**Ahlstrom, E. H., K. Amaoka, D. A. Hensley, H. G. Moser, and B. Y. Sumida.**
**1984.** Pleuronectiformes: development. *In* H. G. Moser, W. J. Richard, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, (eds.), Ontogeny and systematics of fishes, p. 640–670. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

**Amaoka, K.**
**1969.** Studies on the sinistral flounders found in the waters around Japan: taxonomy anatomy and phylogeny. J. Shimonoseki Univ. Fish. 18:65–40.

**Batts, B. S.**
**1964.** Lepidology of the adult pleuronectiform fishes of the Puget Sound, Washington. Copeia. 1964(4):666–673.

**Borets, L. A.**
**1983.** A new species of flounder, *Microstomus shuntovi* sp. n. (Pleuronectidae), and two rare flounder species (Bothidae) from seamounts of the Northwestern and Hawaiian ridges. J. Ichthyol. 23(5):1–6.

**Brooks, D. R., and D. A. McLennan.**
**1991.** Phylogeny, ecology, and behavior: a research program in comparative biology. Univ. Chicago Press, Chicago, IL, 434 p.

**Chapleau, F.**
**1993.** Pleuronectiform relationships: a cladistic reassessment. Bull. Mar. Sci. 52(1):516–540.

**Chapleau, F., and A. Keast.**
**1988.** A phylogenetic reassessment of the monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces; Pleuronectiformes). Can. J. Zool. 66: 2797–2810.

**Chiu, T.-S.**
**1990.** Inference on the phylogenetic relationships of the genus *Glyptocephalus* (Pleuronectiformes: Pleuronectidae) by shape. Bull. Inst. Zool., Academia Sinica 29(2):95–104.

**Cooper, J. A., and F. Chapleau.**
**1998.** Phylogenetic status of *Paralichthodes algoensis* (Paralichthodidae). Copeia 1998(2):477–481.

**Cuvier, G.**
**1816.** Le règne animal distribué d'après son organisation pour servir de base a l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Les reptiles, les poissons, les mollusques et les annelides, edition 1, vol. 2, 532 p.

**de Groot, S. J.**
**1971.** On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). Neth. J. Sea Res. 5(2):121–196.

**Eschmeyer, W. N.**
**1990.** Catolog of the genera of the recent fishes. California Academy of Sciences, San Francisco, CA, 697 p.

**FAO (Food and Agriculture Organization).**
**1997.** FAO yearbook, fishery statistics-catches and landings 1995, vol. 80. FAO, Rome, 714 p.

**Felsenstein, J.**
**1978.** The number of evolutionary trees. Syst. Zool. 27: 27–33.

**Forey, P. L., C. J. Humphries, I. J. Kitching, R. W. Scotland, D. J. Siebert, and D. M. William.**
**1992.** Cladistics: a practical course in systematics. Clarendon Press, Oxford, 191 p.

**Fukuhara, O.**
**1988.** Morphological and functional development of larval and juvenile *Limanda yokohamae* (Pisces: Pleuronectidae) reared in the laboratory. Mar. Biol. 99:271–281.

**Goloboff, P. A.**
**1991.** Homoplasy and the choice among cladograms. Cladistics 1991(7):215–232.

**Hart, J. L.**
**1973.** Pacific fishes of Canada. Fish. Res. Board Can., Bulletin 180, Ottawa, 740 p.

**Hensley, D. A.**
**1993.** Two new flatfish records from the Red Sea, an Indo-Pacific samarid (*Samariscus inornatus*) and the European plaice (*Pleuronectes platessa*). Israel J. Zool. 39:371–379.

**Hensley, D. A., and E. H. Ahlstrom.**
**1984.** Pleuronectiformes: relationships. *In* H. G. Moser, W. J. Richard, D. M. Cohen, M. P. Fahay, A. W. Kendall Jr., and S. L. Richardson, (eds.) Ontogeny and systematics of fishes, p. 670–687. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

**Hubbs, C. L.**
**1945.** Phylogenetic position of the Citharidae, a family of flatfishes. Misc. Pub. Museum Zool. Univ. Mich. 63:1–38.

**Hubbs, C. L., and K. F. Lagler.**
**1970.** Fishes of the Great Lakes region. Univ. Michigan Press, Ann Arbor, MI, 213 p.

**Jordan, D. S.**
**1923.** A classification of fishes, including families and genera as far as known. Stanford Univ. Publ., Biol. Sci. 3(2):79–243.

**Jordan, D. S., and D. K. Goss.**
**1889.** A review of the flounder and soles (Pleuronectidae) of America and Europe. Rep. U.S. Com. Fish. Washington 14(1886):225–342.

**Jordan, D. S., and B. W. Evermann.**
**1898.** The fishes of North and Middle America III. Bull. U.S. Nat. Mus. 47(3): 2602–2712.

**Jordan, D. S., and E. C. Starks.**
**1906.** A review of the flounders and soles of Japan. Proc. of the USNM. 31:161–246.

**Leipertz, S. L.**
**1987.** Morphometrics and the evolutionary history of fishes of the teleost subfamily Pleuronectinae. Ph.D. diss., Univ. Washington, Seattle, WA, University Microfilm International Microfilm No. 8802282.

**Leviton, A. E., R. H. Gibbs Jr., E. Heal, and C. E. Dawson.**
**1985.** Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985(3):802–832.

**Maddison, W. P., M. J. Donoghue, and D. R. Maddison.**
**1984.** Outgroup analysis and parsimony. Syst. Zool. 33:83–103.

**Maddison, W. P., and D. R. Maddison.**
**1992.** MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer Sunderland, 398 p.

**Markle, D. F., P. M. Harris and C. L. Toole.**
**1992.** Metamorphois and an overview of early-life-history stages in Dover sole *Microstomus pacificus*. Fish. Bull. 90:285–301.

**Nelson, J. S.**
**1984.** Fishes of the world, 2nd ed. John Wiley & Sons, New York, NY, 523 p.
**1994.** Fishes of the world, 3rd ed. John Wiley & Sons, New York, NY, 600 p.

**Norman, J. R.**
**1934.** A systematic monograph of the flatfishes (Heterosomata), vol. 1: Psettodidae, Bothidae, Pleuronectidae. British Museum (Natural History), London, 459 p.

**Pertseva-Ostroumova, T. A.**
**1961.** The reproduction and development of far eastern flounders (Pleuronectidae). T. Inst. Okeanol. Akak. Nauk. SSSR. [In Russian; transl. avail. Fish. Res. Board Canada, Transl. Serv. No. 856. 1967.]

**Rass, T. S.**
**1996.** On taxonomy of Pleuronectini (Pleuronectidae). J. Ichthyol. 36(7):546–548.

**Regan, C. T.**
**1910.** The origin and evolution of the teleostean fishes of the order Heterosomata. Ann. Mag. Nat. Hist. 8(6):484–496.
**1920.** A revision of the flat-fishes (Heterosomata) of Natal. Ann. Durban Mus. 2:205–222.
**1929.** Fishes. Article *in* Encyclopaedia Britannica, 14th ed., vol. 19, p. 324–325.

**Ride, W. D. L., C. W. Sabrosky, G. Bernardi, and R. V. Melville.**
**1985.** International code of zoological nomenclature. International Trust for Zoological Nomenclature, British Museum of Natural History, London, 338 p.

**Robins, C. R., R. M. Bailey, C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea, and W. B. Scott.**
**1991.** Common and scientific names of fishes from the United States and Canada, fifth ed., 1991. Am. Fish. Society Spec. Publ. 20, 183 p.

**Roff, D. A.**
**1981.** Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket? Can. J. Fish. Aquat. Sci. 38:968–977.

**Rohlf, F. J.**
**1982.** Consensus indices for comparing classifications. Math. Biosci. 59:131–144.

**Sakamoto, K.**
**1984a.** Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes). Mem. Fac. Fish. Hokkaido Univ. 31:95–215.
**1984b.** Family Pleuronectidae. *In* H. Masuda, K. Amaoka, C. Arai, T. Uyeno, and T. Yoshino (eds.), The fishes of the Japanese Archipelago, p. 351–354. Tokai Univ. Press, Tokyo.

**Sanderson, M. J., and M. J. Donoghue.**
**1989.** Patterns of variations in levels of homoplasy. Evolution 43(8):1781–1795.

**Scott, W. B., and M. G. Scott.**
**1988.** Atlantic fishes of Canada. Can. Bull. Fisheries and Aquatic Sciences 219. Univ. Toronto Press, Canada, 731 p.

**Stiassny, M. L. J., and M. C. C. de Pinna.**
**1994.** Basal taxa and the role of cladistic patterns on the evaluation of conservation priorities: view from freshwater. *In* P. L. Forey, C. J. Humphries, and R. I. Vane-Wright (eds.), Systematics and conservation evaluation, Systematics Association special vol. 50, p. 235–249. Clarendon Press, Oxford.

**Watrous, L. E., and Q. D. Wheeler.**
**1981.** The outgroup comparison method of character analysis. Syst. Zool. 30:1–11.

**Wheeler, A.**
**1992** A list of the common and scientific names of fishes of the British Isles. J. Fish Biol. 41(suppl. A), p. 16, 22.

**Wiley, E. O.**
**1981.** Phylogenetics: the theory and practice of phylogenetic systematics. Wiley-Interscience Publications, John Wiley and Sons, Toronto, 439 p.

**Yazdani, G. M.**
**1969.** Adaptation in the jaws of flatfish (Pleuronectiformes). J. Zool. (Lond.) 159:181–222.

**Yusa, T.**
**1960.** Differences of structures of eggs and larvae between *Limanda yokohamae* Günther and *Limanda schrenki* Schmidt. Bull. Mar. Biol. Stn. Asamushi 10(2):127–131.

## Appendix

### List of characters

1 *Attachment of ocular-side frontal with mesethmoid.* 2 states, 1 step, cci=1.00: frontal of ocular side not in contact with mesethmoid, separated by prefrontal (state 0), frontal of ocular side in contact with mesethmoid (state 1), (see Fig. 104, p. 184 in Amaoka, 1969).

2 *Sensory canal of ocular-side preorbital (Sakamoto, 1984a).* 2 states, 3 steps, cci=0.33: present (state 0), absence (state 1). The presence or absence of a sensory canal in the ocular-side preorbital in the outgroup taxa *Psettodes* sp. could not be determined (state ?). A sensory canal is common in left-eyed flounders, (see Fig. 105, p. 187, in Amaoka, 1969).

3 *Morphology of metapterygoid.* 2 states, 2 steps, cci=0.50: ventral margin concave (state 0, Fig. 2A), observed in *Reinhardtius stomias* and all outgroup taxa. Ventral margin not concave nearly straight (state 1, Fig. 2, B–D).

4 *Articulation between first and second basibranchials.* 2 states, 8 steps, cci=0.13: wedgelike articulation between first and second basibranchial (state 0, Fig. 3A). No wedgelike articulation, first basibranchial loosely attached to second basibranchial by cartilage (state 1, Fig. 3, B–D).

5 *Articulation suture between second and third basibranchials.* 2 states, 3 steps, cci=0.33: wedgelike articulation between second and third basibranchial (state 0, Fig. 3A). No wedgelike articulation, second basibranchial loosely attached to third basibranchial by cartilage (state 1, Fig. 3, B–D).

6 *Haemapophysis through fusion of parapophysis of posterior most abdominal vertebrae (Sakamoto, 1984a).* 2 states, 4 steps, cci=0.25: present (state 0, Fig. 4, A, B, and D), absent (state 1, Fig. 4C).

7 *Accessory processes on ventral side of centrum.* 2 states, 5 steps, cci=0.20: present (state 0, Fig. 5A), absent (state 1, Fig. 5, B and C).

8 *Infraorbitals on ocular side (Sakamoto, 1984a).* 2 states, 2 steps, cci=0.50: absent (state 0), present (state 1). All pleuronectid taxa (except *Microstomus bathybius*) have infraorbital bones on the ocular side. This character state is shared with *Psettodes* and *Paralichthys lethostigmus.* The sensory canal bones are absent in other outgroup taxa examined (*Lepidoblepharon ophthalmolepis*, *Citharichthys arenaceus*, and *Paralichthys squamilentus*), and most taxa within the bothoid lineage, (see Table 6, p. 193 in Amaoka, 1969).

9 *Oil globules in yolk (Hensley and Ahlstrom, 1984).* 2 states, 3 steps, cci=0.33: present (state 0), absent (state 1). All outgroup taxa have at least one oil globule (Hensley and Ahlstrom, 1984).

10 *Morphology of olfactory lamellae (Norman, 1934).* 2 states, 2 steps, cci=0.50: olfactory lamellae radiating around a central rachis (state 0). Olfactory lamellae parallel without a central rachis (state 1).

11 *Morphology of anterior prootic foramen, on ocular side.* 2 states, 8 steps, cci=0.13: sphenotic forms dorsal margin of foramen (state 0, Fig. 7A). Pterosphenoid and prootic join to form dorsal margin of foramen (state 1, Fig. 7, B and C).

12 *Bifurcation of first epibranchial (Sakamoto, 1984a).* 2 states, 5 steps, cci=0.20: distal end of first epibranchial divided into two branches (state 0, Fig. 3, A and B), distal end of first epibranchial simple (state 1, Fig. 3, C and D).

13 *Spines or teeth on gill rakers (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

14 *Structure of suture between mesethmoid and blind-side prefrontal.* 3 states, ordered, 13 steps, cci=0.15: anterior margin of upper orbit is incomplete, mesethmoid and prefrontal on blind side not completely overlapping resulting in a space between them (state 0, Fig. 8A). Anterior margin on upper orbit is complete, mesethmoid and prefrontal on blind side completely overlapping or sutured with a small foramen present (state 1, Fig. 8, B and C). Same as state 1 but foramen absent (state 2, Fig. 8D). The character state for the mesethmoid and prefrontal is not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* owing to the morphological differences in this region with respect to the ingroup taxa.

15 *Structure of first anal ptergyiophore.* 2 states, 3 steps, cci=0.33: thin (state 0). Broadly thickened (state 1).

16 *Uniformity of dentition.* 3 states, ordered, 6 steps, cci=0.33: dentition of variable lengths and size (state 0, Fig. 9A). Dentition of near equal or equal length (state 1, Fig. 9, B and C). Uniform dentition as in state 1 but teeth forming a continuous cutting edge (state 2, Fig. 9D).

17 *Development of interorbital process (Sakamoto, 1984a).* 3 states, unordered, 4 steps, cci=0.50: well developed (state 0, Fig. 10, A and C). Middle part absent (state 1). Not developed (state 2, Fig. 10, B, D, and E).

18 *Structure of hyomandibular of both ocular and blind sides.* 2 states, 2 steps, cci=0.50: anterior margin of hyomandibular is not broad (state 0, Fig. 2, A–C). Anterior margin of hyomandibular is flatly broadened (state 1, Fig. 2D).

19 *Dentition of third epibranchial (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

20 *Bony plates of the branchial arch (Sakamoto, 1984a).* 2 states, 2 steps, cci=0.50: present (state 0, Fig. 3, A and B), absent (state 1, Fig. 3, C and D).

21 *Number of rows of gill rakers on fourth ceratobranchial.* 3 states, unordered, 6 steps, cci=0.33: one row, second row absent or very reduced (state 0, Fig. 3, A and B). Zero rows (state 1). Two rows (state 2, Fig. 3, C and D). *Reinhardtius* may have 1 row of gill rakers on fourth ceratobranchial, but very modified and difficult to distinguish from bony plates.

22 *Fimbriation along posterior dorsal margin of the operculum.* 2 states, 2 steps, cci=0.50: absent (state 0, Fig. 2A), present (state 1, Fig. 2, B–D).

23 *Number of abdominal vertebrae.* 3 states, ordered, 9 steps, cci=0.22: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). Less than 12 (state 0). Between 12 and 14 (state 1). Greater than 15 (state 2).

24 *Shape of caudal fin.* 2 states, 2 steps, cci=0.50: caudal fin truncate, double truncate or rounded (state 0). Caudal fin lunate or emarginate (state 1).

25 *Position of migrated eye in relation to dorsal midline (Sakamoto, 1984a).* 2 states, 3 steps, cci=0.33: located on side of head (state 0). Near dorsal midline, visible on blind side (state 1).

26 *Increase in number of caudal vertebrae.* 3 states, ordered, 5 steps, cci=0.40: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). Less than or equal to 35 (state 0). From 36 to 41 (state 1). Greater than 41 (state 2).

27 *Foramen on blind-side dentary just below margin of dentition.* 2 states, 5 steps, cci=0.20: present (state 0), absent (state 1).

28 *Attachment of palatine with ocular-side pterygoid.* 2 states, 2 steps, cci=0.50: palatine and pterygoid attached (state 0, Fig. 2, A and D). Palatine and pterygoid not attached due to a reduction of the posterior ventral arm of the palatine (state 1, Fig. 2, B and C).

29 *Gill rakers on first epibranchial.* 2 states, 2 steps, cci=0.50: present (state 0, Fig. 3, A–D), absent (state 1).

30 *Gill rakers on second epibranchial.* 3 states, ordered, 5 steps, cci=0.40: present (state 0, Fig. 3, B and C). Reduced to just one gill raker at proximal base of epibranchial (state 1, Fig. 3, A and D). Absent (state 2).

31 Gill rakers on third epibranchial. 3 states, ordered, 5 steps, cci=0.40: present (state 0, Fig. 3B). Reduced to just one gill raker at proximal

base of epibranchial (state 1, Fig. 3D). Absence (state 2, Fig. 3C).

32 *Gill rakers on first hypobranchial.* 2 states, 4 steps, cci=0.25: present (state 0, Fig. 3, A–D). Reduced to just one gill raker at distal base of hypobranchial (state 1).

33 *Subdivisions of hypurals or hypurals and parhypurals (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1). Character states for the caudal skeleton is not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* owing to the morphological differences in this region with respect to the ingroup taxa.

34 *Articulation of blind-side metapterygiod with entopterygoid.* 2 states, 7 steps, cci=0.14: absent (state 0, Fig. 2, A and B), present (state 1, Fig. 2, C and D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Fig. 2A).

35 *Morphology of mesethmoid and prefrontal on blind side, special type for Verasper.* 2 states, 1 step, cci=1.00: large open foramen absent (state 0). Large open formamen formed between mesethmoid and prefrontal on blind side (state 1), autapomorphy for *Verasper*. The character state for the mesethmoid and prefrontal is not applicable (na) for the outgroup taxa *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* owing to the morphological differences in this region with respect to the ingroup taxa.

36 *Gill rakers on second hypobranchial.* 2 states, 4 steps, cci=0.25: present (state 0, Fig. 3, A and C). Reduced to just one gill raker at distal base of hypobranchial (state 1, Fig. 3, B and D).

37 *Sphenotic process in relation to hyomandibular socket.* 2 states, 3 steps, cci=0.33: process position low on sphenotic to form dorsal roof of socket between sphenotic and prootic (state 0, Fig. 7A). Process is positioned higher on sphenotic and more anteriorly so as not to be associated with socket (state 1, Fig. 7, B and C).

38 *Supraoccipital crest.* 2 states, 7 steps, cci=0.14: single crest (state 0, Fig. 10, A and B). Double crest to form a groove for the insertion of the basal ends of pterygiophores for the dorsal fin (state 1, Fig. 10, C–E).

39 *Cardiac apophysis of urohyal (Sakamoto, 1984a).* 3 states, unordered, 8 steps, cci=0.25: cardiac apophysis is simple or slightly bifurcate at the posterior portion (state 0, Fig. 12, A and B) Cardiac apophysis is simple at tip with bifurcation positioned anteriorly on dorsal edge (state 1, Fig. 12, E and F). Cardiac apophysis is strongly bifurcate at the posterior portion (state 2, Fig. 12, C and D).

40 *Gill rakers on fourth epibranchial.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 3, A–D), present (state 1).

41 *Rows of teeth in lower jaw (Norman, 1934).* 2 states, 6 steps, cci=0.17: multiserial or biserial rows of teeth (state 0). Uniserial row of teeth (state 1). The distribution from published accounts of this character was reexamined in the specimens used for this analysis. The state that was observed in these specimens was used if the number of rows was in conflict with the published accounts in Norman (1934).

42 *Barbed teeth (Norman, 1934).* 2 states, 2 steps, cci=0.50: absent (state 0), present (state 1).

43 *Supratemporals on ocular side.* 2 states, 7 steps, cci=0.1: supratemporal in one piece (state 0, Fig. 13A). Supratemporal in two pieces, jointed at anterior bifurcation point (state 1, Fig. 13B).

44 *Supratemporals on blind side.* 2 states, 5 steps, cci=0.20: Supratemporal in one piece (state 0, Fig. 13A). Supratemporal in two pieces, jointed at anterior bifurcation point (state 1, Fig. 13B).

45 *Scales on the surface eyes (Sakamoto, 1984a).* 2 states, 6 steps, cci=0.17: absent (state 0), present (state 1).

46 *Posterior region of supraoccipital.* 2 states, 2 steps, cci=0.50: presence of a flat plate triangular or diamond shaped extending posteriorly from supraoccipital between or overtop epiotics (state 0, Fig. 10, A, and C–E). Absence of posterior extension of supraoccipital (state 1, Fig. 10B).

47 *Anterior prootic foramen on blind side.* 2 states, 8 steps, cci=0.13: sphenotic forms dorsal margin of foramen (state 0, 7A). Pterosphenoid and prootic join to form dorsal margin of foramen (state 1, Fig. 7, B and C). The anterior prootic foramen entirely contained within prootic in outgroup taxa.

48 *Pterosphenoid on blind side.* 2 states, 3 steps, cci=0.33: pterosphenoid reduced, frontal and parasphenoid join to form posterior margin of orbit (state 0, Fig. 7B). Pterosphenoid large forming posterior margin of orbit (state 1, Fig. 7, A and C).

49 *Rows of teeth on fifth ceratobranchial.* 3 states, ordered 4 steps, cci=0.75: irregular single or multiple rows (state 0, Fig. 3A). Regular 2 rows (state 1, Fig. 3, B and C). Regular multiple rows or clustered (state 2, Fig. 3D).

50 *Nasal bone on blind side.* 2 states, 7 steps, cci=0.14: present (state 0), absent (state 1). The presence of a blind-side nasal bone could not be determined (state ?) for the outgroup taxa *Citharichthys arenaceus* either through cleared and stained material or through radiograph.

51 *Number of infraorbital bones on blind side (Sakamoto, 1984a).* 3 states, unordered 9 steps, cci=0.22: from five to seven (state 0). Greater than seven (state 1). Less than five (state 2). The number of infraorbital bones in *Citharichthys arenaceus* could not be determined (state ?) owing to the poor preservation of infraorbital bones for this specimen.

52 *Dorsal crest extending anteriorly from supraoccipital to blind-side frontal.* 2 states, 7 steps, cci=0.14: prominent narrow crest present, angled laterally to ocular side (state 0, Fig. 10, B and C). Reduced of absent crest resulting in a flattened posterior dorsal region on blind-side frontal (state 1, Fig. 10, A, D, and E).

53 *Anterior margin of mesethmoid.* 3 states, unordered, 10 steps, cci=0.30: open canal extending from frontal on ocular side (state 0, Fig. 8A). Thin plate (state 1, Fig. 8B). Closed canal extending from frontal on ocular side or from interorbital process if present. Process extending anteriorly with a triangular shape to make anterior edge thicker (state 2, Fig. 8C). Similar to state 2 but anterior edge reduced to form an irregular edge (state 3, Fig. 8D). The morphology of the mesethmoid in the outgroup taxa, *Psettodes* sp. and *Lepidoblepharon ophthalmolepis* is unlike any observed in the other outgroup taxa or within the ingroup and is coded as not applicable (na).

54 *Articulation of metapterygoid with ocular-side entopterygoid.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 2, A–C), present (state 1, Fig. 2D). The metapterygoid is articulated to the pterygoid in outgroup taxa (Fig. 2A).

55 *Dentary fossa for the insertion of Meckel's cartilage on both ocular- and blind-side dentaries.* 2 states, 1 steps, cci=1.00. Present (state 0, Fig. 9, A and B), absent (state 1, Fig. 9, C and D).

56 *Ceratohyal foramen (Sakomoto, 1984a).* 2 states, 1 step, cci=1.00: present (state 0), absent (state 1).

57 *Intercalar in contact with basioccipital.* 2 states, 4 steps, cci=0.25: intercalar not joined to basioccipital (state 0, Fig. 7, A and B). Intercalar and basioccipital in contact (state 1, Fig. 7C).

58 *Posterior extension of supratemporal branch of lateral line (Norman, 1934).* 2 states, 4 steps, cci=0.25: absent (state 0), present (state 1).

59 *Prolongation of anterior dorsal-fin rays (Norman, 1934; Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1).

60 *Rows of teeth in upper jaw (Norman, 1934).* 2 states, 3 steps, cci=0.33: multiserial or biserial rows of teeth (state 0). Uniserial row of teeth (state 1). Character state based on direct observation of materials mantained priority in observations conflicting with Norman (1934).

61 *Asymmetry in medial symphysis of premaxillae.* 2 states, 1 step, cci=1.00: blind-side premaxilla does not protrude past sagittal axis at anterior symphysis with ocular-side premaxilla (state 0, Fig. 9, A and B). Blind-side premaxilla overlaps or protrudes past sagittal axis (state 1, Fig. 9, C and D).

62 *Asymmetry in length of premaxillae.* 2 states, 2 steps, cci=0.50: ocular-side nearly equal or equal to length of blind-side premaxilla (state 0, Fig. 9, A and B). Ocular-side premaxilla shorter than blind-side premaxilla (state 1, Fig. 9, C and D).

63 *Ventral posterior curvature of blind-side premaxilla.* 2 states, 2 steps, cci=0.50: absent (state 0, Fig. 9, A and B), present (state 1, Fig. 9, C and D).

64 *Asymmetry of size of space along ventral margin of dentary and articular.* 2 states, 5 steps, cci=0.20: space nearly the same size (state 0, Fig. 9, A, B, and D). Asymmetrical space, blind side greater than ocular side (state 1, Fig. 9C).

65 *Asymmetry in size of dorsal posterior process of dentary.* 2 states, 5 steps, cci=0.20: symmetrical (state 0, Fig. 9, B and D). Asymmetrical, ocular side greater than blind side (state 1, Fig. 9, A and C).

66 *Reduction of teeth on ocular-side maxilla (Norman, 1934).* 3 states, ordered, 5 steps, cci=0.40: number of teeth on ocular side nearly equal or equal to those on blind side (state 0, Fig. 9, A and B). Ocular-side teeth fewer than blind- side teeth but greater than 6 (state 1, Fig. 9C). Ocular-side teeth fewer than blind-side teeth, less than 6 (state 2, Fig. 9D).

67 *Reduction of teeth on ocular-side dentary (Norman, 1934).* 3 states, ordered 6 steps, cci=0.33: number of teeth on ocular side nearly equal or equal to those on blind side (state 0, Fig. 9, A and B). Ocular-side teeth fewer than blind-side teeth but greater than 6 (state 1, Fig. 9C). Ocular-side teeth fewer than blind-side teeth , less than 6 (state 2, Fig. 9D).

68 *Epiotic process on ocular and blind sides.* 2 states, 3 steps, cci=0.33: process absent in *Microstomus pacificus* or barely evident in *Hippoglossoides* and *Reinhardtius* (state 0, Fig. 10, A–C). Process is clearly evident extending anteriorly from epiotics onto parietals in *Limanda* and *Platichthys* (state 1, Fig. 10, D and E).

69 *Asymmetry in size of entopterygoid.* 2 states, 3 steps, cci=0.3: blind side equal in size to ocular side (state 0, Fig. 2, A and B). Blind side smaller than ocular side (state 1, Fig. 2, C and D).

70 *Shape of teeth (Norman, 1934).* 4 states, ordered 6 steps, cci=0.50: sharply pointed (state 0, Fig. 9, A and B). Bluntly conical or with truncated tips (state 1, Fig. 9C). Incisorlike (state 2, Fig. 9D). Molariform (state 3).

71 *Shape of fifth ceratobranchial.* 3 states, ordered 5 steps, cci=0.40: straight "rod" shaped (state 0, Fig. 3, A and B). Slight curve on medial margin found in *Limanda, Parophrys, Psettichthys, Isopsetta* etc. (state 1, Fig. 3C). Strong curve on medial margin, the ceratobranchials forming or almost forming a triangular plate found in *Pleuronectes* and *Platichthys* (state 2, Fig. 3D).

72 *Dentition profile on fifth ceratobranchial.* 3 states, ordered, 6 steps, cci=0.33: pointed (state 0, Fig. 3, A and B). Bluntly pointed (state 1, Fig. 3C). Rounded or molariform (state 2, Fig. 3D).

73 *Structure of haemal spines in anteriormost caudal vertebrae.* 2 states, 6 steps, cci=0.17: broadly attached both anteriorly and posteriorly to centrum, lateral foramen in haemal arch present (state 0, Fig. 5B). Narrow at base, attached anteriorly to centrum, lateral foramen in haemal arch absent (state 1, Fig. 5, A and C).

74 *Structure of epiotics (Sakamoto, 1984a).* 2 states, 2 steps, cci=0.50: epiotics not joined to each other along dorsal posterior margin of skull (state 0). Epiotics joined (state 1), only observed in *Isopsetta isolepis, Microstomus pacificus.*

75 *Egg type (Hensley and Ahlstrom, 1984).* 2 states, 2 steps, cci=0.50: pelagic egg (state 0). Demersal egg (state 1).

76 *Thickened lips (Norman, 1934).* 2 states, 2 steps, cci=0.50: absent, thin lips (state 0), present, lips thickened (state 1). Taxa in Pleuronectini are also described as having thickened lips (Norman, 1934) but this is not as evident as in Microstomini.

77 *Structure of exoccipital and prootic.* 2 states, 2 steps, cci=0.50: exoccipital and prootic joined (state 0, Fig. 7B). Exoccipital and prootic not joined (state 1, Fig. 7, A and C).

78 *Postocular ridge on ocular side (Norman, 1934).* 2 states, 2 steps, cci=0.50: absent (state 0), present (state 1).

79 *Villiform teeth (Norman, 1934).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1).

80 *Foramen in blind-side prefrontal.* 2 states, 2 steps, cci=0.50: absent (state 0), present (state 1).

81 *Lateral process on ocular-side frontal.* 2 states, 3 steps, cci=0.33: absent (state 0, Fig. 7, A and C, Fig. 10, A, B, D, and E), present (state 1, Fig. 7B, Fig. 10C).

82 *Decrease in the number of caudal vertebrae.* 2 states, 3 steps, cci=0.33: counts for Pleuronectidae taken from Sakamoto (1984a, Table 13). Greater than 25 (state 0). Less than or equal to 25 (state 1). The polarity established for this character is an exception to the general outgroup comparison methodology. It is apparent that a caudal vertebrae count between 25 and 35 is plesiomorphic for Pleuronectidae and for many taxa within the bothoid lineage. Despite the heterogeneity in *Citharichthys arenaceus* and less than 25 vertebrae observed in *Lepidoblepharon ophthalmolepis* and *Psettodes* sp.; the reduction of caudal vertebrae observed within Pleuronectidae is considered a derived character state.

83 *Pterosphenoid on ocular side* . 2 states, 2 steps, cci=0.50: pterosphenoid reduced, frontal and parasphenoid join to form posterior margin of orbit (state 0, Fig. 7B). Pterosphenoid large and forms posterior margin of orbit (state 1, Fig. 7, A and C).

84 *Position and origin of dorsal fin (Norman, 1934; Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: originating nearly on the median line of head, ancestral (state 0). Originating on blind side (state 1).

85 *Cartilagenous interspace between blind-side prefrontal and parasphenoid.* 2 states, 1 step, cci=1.00: present (state 0, Fig. 7, A and C), reduced or absent (state 1, Fig. 7B).

86 *Mesethmoid in relation to upper orbit (Sakamoto, 1984a).* 3 states, ordered, 3 steps, cci=1.00: completely forms anterior margin of orbit (state 0). Forms only part of anterior margin in orbit (state 1). Does not form part of orbit (state 2).

87 *Extension of intestine into body cavity (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), presence (state 1).

88 *Number of caudal-fin rays.* 5 states, ordered, 7 steps, cci=0.57: the number of caudal-fin rays was taken as the modal value for counts in Sakamoto (1984a, Table 18). 18 or 19 (state 0), 20 (state 1), 21 (state 2), 22 (state 3), 23 (state 4).

89 *Degree of caudal-fin ray branching.* 3 states, unordered, 8 steps, cci=0.25: expressed as the number of caudal-fin rays minus the number of branched caudal-fin rays. Six unbranched rays (state 0). Less than 6 unbranched rays (state 1). Greater than 6 unbranched rays (state 2).

90 *Position of ventral anterior tip of pelvis of ocular side in relation to cleithra (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: pelvis posterior to cleithrum ancestral (state 0), observed in majority of outgroup as well as Pleuronectidae . Pelvis anterior to cleithrum autapomorphy for *Microstomus bathybius* (state 1).

91 *Structure of scales (Batts, 1964).* 2 states, 1 step, cci=1.00: radii extending anteriorly from focus of each scale (state 0). Radii completely surrounding focus of scale (state 1).

92 *Fimbriation along the posterior ventral margin of the interoperculum.* 2 states, 1 step, cci=1.00: absent (state 0, Fig. 2, A, B, and D), present (state 1, Fig. 2C).

93 *Fimbriation along posterior margin of the suboperculum.* 2 states, 2 steps, cci=0.50: absent (state 0, Fig. 2, A, B, and D), present (state 1, Fig. 2C).

94 *Relation of cleithra with urohyal (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: cleithra not inserted by tip of urohyal (state 0), most common state in bothoid group. Cleithra slightly inserted by tip of urohyal (state 1).

95 *Increase in the number of pyloric appendages on upper intestine (Norman, 1934).* 2 states, 1 step, cci=1.00: two or three pyloric appendages plus one on upper intestine (state 0). Two to four pyloric appendages plus two or three on upper intestine (state 1).

96 *Asymmetry in nasal bones (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: ocular side larger than blind side with blind side reduced or absent as recorded in character 50 (state 0). Blind side larger than ocular side (state 1). States could not be observed (state ?) in the outgroup taxa *Psettodes* sp., *Citharichthys arenaceus*, and *Paralichthys squamilentus* owing to the poor condition of the specimens around the dorsal anterior margin of the upper eye. A blind-side nasal bone was not observed in radiographs of these specimens prior to clearing and staining.

97 *Mucous cavities on blind side of head (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1).

98 *Interpterosphenoid bar.* 2 states, 7 steps, cci=0.14: absent (state 0), present (state 1). The interpterosphenoid bar is a medial extension of the pterosphenoid foramen. This morphological character can be observed only from a lateral view, through the pterosphenoid if it is thin, or from a ventral view if the parasphenoid is removed (Fig. 16).

99 *Foramen on ocular-side dentary just below margin of dentition.* 2 states, 11 steps, cci=0.09: present (state 0), absent (state 1).

100 *Post-ocular ridge on blind side.* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1).

101 *Bony prominences along ocular-side postocular ridge.* 3 states, unordered, 4 steps, cci=0.50: bony prominences absent (state 0, Fig. 10D). Many small bony prominences (state 1, Fig. 10E). A series of enlarged prominences on postocular ridge in *Pleuronectes platessus* and *P. quadrituberculatus* (state 2).

102 *Bony prominences along blind-side postocular ridge.* 2 states, 3 steps, cci=0.33: bony prominences absent (state 0, Fig. 10D). Many small bony prominences present (state 1, Fig. 10E).

103 *Bony prominences extending anteriorly onto interorbital bar.* 2 states, 1 step, cci=1.00: prominences not extended anteriorly (state 0). Prominences extending anteriorly onto interorbital bar of the ocular-side frontal (state 1, Fig. 10E).

104 *Scales on median-fin rays (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1). The presence of scales on median-fin rays in the outgroup taxa *Paralichthys squamilentus* could not be determined (state ?), because it was apparent that all of the scales on this specimen had been lost during clearing and staining.

105 *Bony plates or tubercles on body (Sakamoto, 1984a).* 2 states, 1 step, cci=1.00: absent (state 0), present (state 1).

106 *Supratemporals fused to cranium (Sakamoto, 1984a).* 2 states, 2 steps, cci=0.5: not fused with cranium (state 0). Fused with cranium (state 1).

# Documenting the bycatch of harbor porpoises, *Phocoena phocoena*, in coastal gillnet fisheries from stranded carcasses

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**Abstract.**—We examined 107 harbor porpoise (*Phocoena phocoena*) carcasses recovered from beaches in Maryland, Virginia, and North Carolina between 1994 and 1996 for evidence of entanglement in fishing gear. Stranded porpoises ranged in length from 102 to 128 cm, indicating that only juvenile porpoises are present in the nearshore waters of the Mid-Atlantic during winter. Of the 40 porpoises for which we could establish cause of death, 25 displayed definitive evidence of entanglement in fishing gear. Evidence of entanglement consisted primarily of line marks from nets; in four cases we were able to determine specifically that porpoises had become entangled in monofilament nets. These mortalities demonstrate the need for a directed observer program for coastal gillnet fisheries in the Mid-Atlantic.

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Large numbers of harbor porpoises (*Phocoena phocoena*) are killed in United States and Canadian commercial fisheries each year. For example, between 1200 and 2900 harbor porpoises were killed annually between 1990 and 1993 in the Gulf of Maine sink gillnet fishery (Bravington and Bisack, 1996). Porpoises from the Gulf of Maine population have also been killed in gillnet fisheries in the Bay of Fundy, where an estimated 424 and 101 porpoises were taken in 1993 and 1994, respectively (Trippel et al., 1996). Efforts are currently underway to reduce mortality in these fisheries by using a variety of mitigation strategies (Resolve[1]).

Harbor porpoises from the Gulf of Maine and Bay of Fundy are believed to constitute a single population (Palka et al., 1996). The population disperses during winter and some individuals move south to the Mid-Atlantic region (defined here as from New York to North Carolina) (Polachek et al., 1995). Strandings of harbor porpoises have been documented as far south as Florida

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[1] Resolve, Inc. 1996. Final draft of the Gulf of Maine/Bay of Fundy harbor porpoise take reduction team take reduction plan. A consensus document prepared by Resolve, Inc., Washington, DC, 33 p.

(Polachek et al., 1995), but the southern limit of the range of the species is generally accepted to be Cape Hatteras, North Carolina (reviewed in Palka et al., 1996). Little is known about the distribution and ecology of this harbor porpoise population in winter; even less is known about interactions with commercial fisheries in the Mid-Atlantic region. Porpoises are taken in a variety of gillnet fisheries in this area (Read, 1994; Read et al., 1996), but to date it has not been possible to determine the full extent or nature of incidental catches in the southern portion of the range of this population. The impact of these incidental takes on the Gulf of Maine and Bay of Fundy population, therefore, is unknown.

Current monitoring efforts for estimating the magnitude of porpoise bycatch in gillnet fisheries in the Mid-Atlantic rely on observers placed aboard commercial fishing vessels operating in this region. The majority of these observed trips take place more than three miles from shore; coverage of nearshore commercial fisheries by observers is extremely limited. An alternative source of information on marine mammal mortality in these nearshore fisheries comes from documentation of recovered stranded carcasses. Here we augment current monitoring programs by reporting observations of stranded harbor porpoises that exhibit evidence of entanglement in the Mid-Atlantic region. Our intent is to provide this information to managers of observer programs, so that they may concentrate efforts in times and areas where entanglements have been documented.

## Methods

Porpoises stranded on the beaches of Maryland, North Carolina, and Virginia[2] in 1994, 1995, and 1996 were examined for evidence of entanglement by using the protocol developed by Haley and Read (1993). This protocol is used by personnel in regional stranding networks to assess the carcasses of stranded marine mammals for the presence of physical evidence consistent with entanglement in fishing gear. Physical evidence of entanglement includes impressions of net material in the epidermis, thin lacerations on appendages, and mutilation, including dismemberment and longitudinal cuts along the ventral abdomen into the body cavity (Fig. 1). In general, these observations are indicative of entanglement and death in fishing gear.

Carcasses were examined on the beach by stranding network personnel for fishery interactions and

[2] Carcasses retrieved in New York, New Jersey, and Delaware were not included in this study because comparable data on fisheries interaction were not available for these specimens.

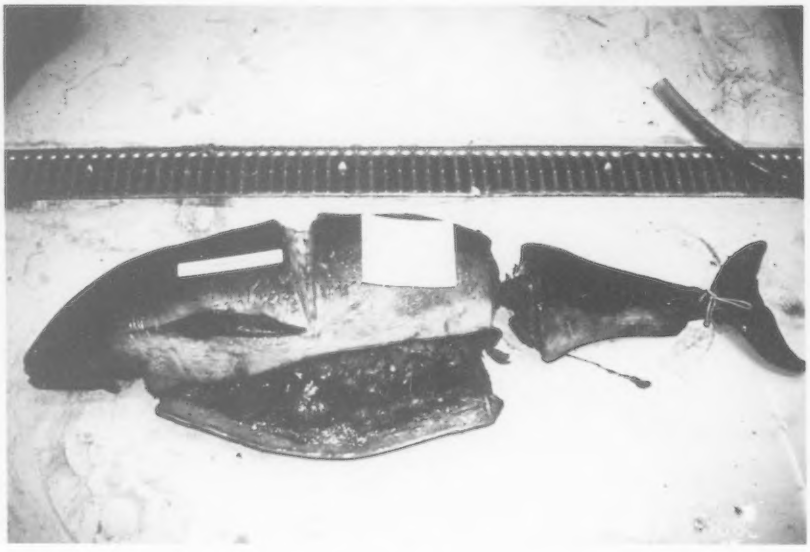

**Figure 1**

Mutilated carcass of a stranded harbor porpoise, *Phocoena phocoena* (MMSC 94-009). Note the net mark encircling the body just anterior to the insertion of the flipper.

were then stored frozen and examined at the Smithsonian Institution (SI) during necropsy workshops in May 1994, November 1995, and October 1996. In all but three specimens, assessments made by stranding network personnel on the beach were compared with independent assessments made at the SI. The determination of entanglement was conservative; an animal was scored as having been entangled only when external lacerations or impressions from net material or when mutilation consistent with entanglement was clearly present. If a carcass was too decomposed to determine cause of death definitively, or if skin was missing from a large proportion of the appendages, it was scored as "CBD" (could not be determined). Read and Murray[3] determined that all porpoises killed in sink gill nets and retrieved by observers exhibited external net marks. Thus, if a carcass could be assessed, but was scored as not having died of entanglement, the animal was assumed to have died of natural causes. Investigators attempted to identify the net type (i.e. monofilament or multifilament) responsible for the entanglement. The condition of the carcass on the SI scale, in which code 1 = alive, code 2 = dead but fresh, code 3 = moderately decomposed, code 4 = severely decomposed, and code 5 = skeletal remains was noted (Geraci and Lounsbury, 1993).

Standard morphometric data (Norris, 1961) were collected and body condition was evaluated as robust (convex epaxial surface and no "neck" evident) or emaciated (concave epaxial surface and "neck" evident) (Kastelein et al., 1997). Testis size and the presence or absence of ovarian scars (Read and Hohn, 1995) were used to determine the sexual maturity of individuals.

A chi-square test (Sokal and Rohlf, 1981) was used to determine whether there was a difference in carcass condition (SI codes 2 and 3) between entangled and nonentangled animals. To determine if there was a difference in body condition (robust or emaciated) in entangled and nonentangled animals, a *G*-test with an adjusted *G*-value for William's correction (Sokal and Rohlf, 1981) was conducted. Two *t*-tests were also used (Sokal and Rohlf, 1981): the first to compare the mean standard lengths of entangled and nonentangled stranded animals; the second to compare the mean standard lengths of stranded entangled animals and 10 nonstranded entangled animals. The standard lengths of the latter set of porpoises were collected by observers working on gillnet vessels off the coasts of Maryland and North Carolina during 1995 and 1996. Only those animals for which we were able to assess evidence of fisheries interaction were included in the statistical analyses; those scored as CBD were excluded from these analyses. Mean standard lengths are reported with associated standard deviations.

[3] Read, A, and K. Murray. 135 Duke Marine Lab Rd., Beaufort, NC 28516. Unpubl. data.

## Results

### Characterization of strandings

We examined 107 harbor porpoise carcasses (Table 1; Fig. 2). We are aware of 17 additional harbor porpoise strandings that occurred between 1994 and 1996 in the Mid-Atlantic but did not include these strandings in our analysis. Fifteen of these 17 carcasses were not examined according to our protocol, and data discrepancies could not be resolved for two others. Of the 107 porpoise carcasses that we included, 104 were examined independently both on the beach and at SI workshops. The remaining three carcasses were examined only on the beach; however, excellent photo-documentation allowed researchers at the SI to confirm the assessments for these three carcasses that had been made on the beach by stranding network personnel.

All harbor porpoise strandings occurred between January and May, with a peak between the months of March and May (Table 2). Fifty-five animals were male, 51 female; the sex of one was unidentifiable

**Table 1**

Summary of harbor porpoises (*Phocoena phocoena*) stranded along the Mid-Atlantic coast (by year and state) and evidence of entanglement. Yes = evidence of fisheries interaction; No = no evidence of fisheries interaction; CBD = evidence of fisheries interaction could not be determined owing to advanced decomposition of carcass.

| Year | State | Total | Yes | No | CBD |
|---|---|---|---|---|---|
| 1994 | NC | 5 | 0 | 2 | 3 |
| | MD | 9 | 4 | 2 | 3 |
| | VA | 39 | 13 | 3 | 23 |
| | Total | 53 | 17 | 7 | 29 |
| 1995 | NC | 7 | 1 | 3 | 3 |
| | MD | 2 | 1 | 1 | 0 |
| | VA | 15 | 0 | 2 | 13 |
| | Total | 24 | 2 | 6 | 16 |
| 1996 | NC | 11 | 2 | 2 | 7 |
| | MD | 3 | 1 | 0 | 2 |
| | VA | 16 | 3 | 0 | 13 |
| | Total | 30 | 6 | 2 | 22 |
| Total | | 107 | 25 | 15 | 67 |

owing to advanced decomposition. The condition of the animals varied: code-3 and code-4 carcasses occurred most frequently in North Carolina and Maryland; the greatest number of code-2 animals was found in Virginia (Table 3; Fig. 3).

We considered 34 animals to be robust and 35 animals emaciated; we could not assess body condition for the remaining 38 animals owing to advanced decomposition. We were able to determine the total standard length of 64 carcasses; 43 were too decomposed to obtain an accurate measurement. Stranded porpoises ranged in length from 102 cm to 128 cm (Mean: 116 ±6 cm; Fig. 4). We were able to assess sexual maturity of 82 porpoises, and all were immature.

## Characterization of fishery interactions

Of the 107 carcasses, we found 15 discrepancies between assessments of fishery interaction made by stranding personnel on the beach and those made at the SI workshops. Fourteen of these carcasses were assessed as entangled on the beach, but at the workshops these carcasses were considered too decomposed to evaluate evidence of entanglement. This deteriorated condition likely resulted from decomposition during transport from the beaches to the SI. Before a final assessment was made, we reviewed photographs and records taken at the initial examination of these 14 carcasses. We concluded from this analysis that four of these porpoises died as a result of entanglement (one of these carcasses, for example, had monofilament net wrapped around it when discovered on the beach). Photographs of ten of these carcasses were inconclusive, therefore, to be conservative, we scored them as "CBD." Another carcass was initially scored on the beach as too decomposed for evaluation, but subsequent assessment at the workshop revealed clear signs of entanglement.

Owing to decomposition of the carcasses, only 40 of the 107 porpoises were assessed for signs of entanglement. Of these, a total of 25 (63%) displayed clear evidence of entanglement (Table 1). Entangled

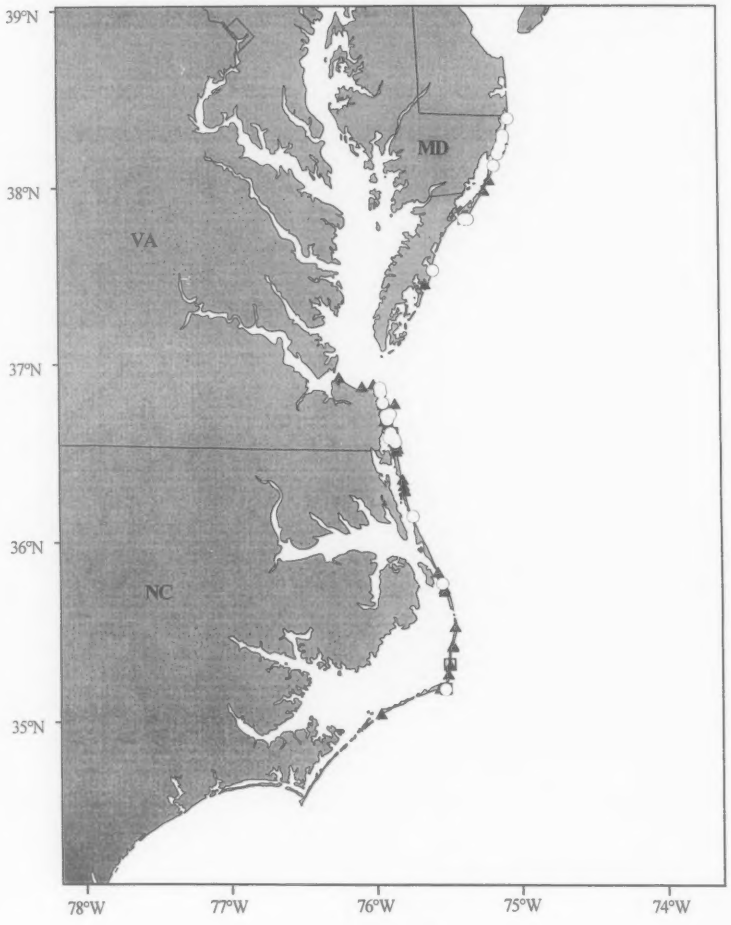


**Figure 2**

Map of the Mid-Atlantic coast showing stranding locations of harbor porpoises, *Phocoena phocoena*, between 1994 and 1996. Open circle = evidence of fishery interaction; open square = no evidence of fishery interaction; closed triangle = evidence of fishery interaction could not be determined due to advanced decomposition of carcass.

**Table 2**

Summary of harbor porpoises (*Phocoena phocoena*) stranded along the Mid-Atlantic coast by month. Yes = evidence of fisheries interaction; No = no evidence of fisheries interaction; CBD = evidence of fisheries interaction could not be determined owing to advanced decomposition of carcass.

| Month | Yes | No | CBD | Total |
|---|---|---|---|---|
| January | 0 | 0 | 1 | 1 |
| February | 2 | 0 | 0 | 2 |
| March | 8 | 7 | 11 | 26 |
| April | 14 | 7 | 39 | 60 |
| May | 1 | 1 | 16 | 18 |
| Total | 25 | 15 | 67 | 107 |

porpoises were observed in all three states between the months of February and May; most ($n$=13) entangled porpoises were found on the beaches of Virginia (Fig. 2; Table 1) in March and April 1994 (Table 2). Twelve entangled animals were male; 13 were female. We were able to positively identify four cases of entanglement in monofilament gill nets.

Nine of the 25 entangled porpoises were fresh (code 2); 14 were moderately decomposed (code 3); two were very decomposed (code 4). The proportion of code-2 and code-3 porpoises in the sample of entangled porpoises was not significantly different from that of nonentangled animals ($\chi^2$=0.82, df=1, $P$=0.36). We were able to assess evidence of entanglement for 93% ($n$=13) of the code-2 animals; 41% ($n$=24) of the code-3 animals; and 9% ($n$=3) of the code-4 animals. Fifteen of the entangled animals were robust, and six were emaciated; four were too decomposed to allow us to determine body condition. The proportion of robust animals in the entangled sample was significantly greater than that in the nonentangled sample ($G_{adj}$=23.51, df=1, $P$<0.001). None of the entangled animals displayed any unusual gross signs of pathology.

The mean total length of entangled animals was 116 ±5 cm (range: 112–128 cm; Fig. 4). The mean total length of entangled porpoises was not significantly different from that of nonentangled animals (116 ±7 cm; $t$=0.396, df=36, $P$=0.69). The mean total length of the entangled stranded animals was significantly less than that of porpoises killed at sea and examined by observers (131 ±14 cm; $t$=2.97, df=9, $P$=0.015).

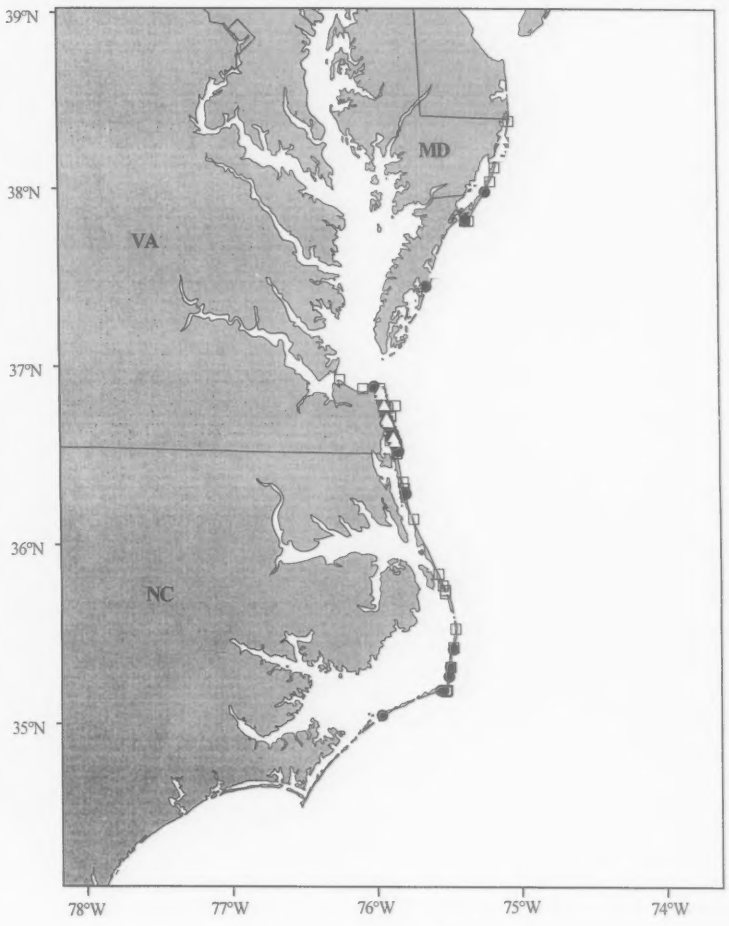


**Figure 3**

Map of the Mid-Atlantic coast showing stranding locations of harbor porpoises, *Phocoena phocoena*, between 1994 and 1996. Open triangle = SI code 2; open square = SI code 3; closed circle = SI code 4.

**Table 3**

Summary of Smithsonian Institution codes to define condition of harbor porpoises (*Phocoena phocoena*) stranded along the Mid-Atlantic coast by state. One animal stranded in Virginia was in code-5 condition. Code 1 = alive; code 2 = dead but fresh; code 3 = moderately decomposed; code 4 = severely decomposed; code 5 = only skeletal remains noted.

| SI Code | NC | VA | MD | Total |
|---|---|---|---|---|
| 2 | 0 | 10 | 4 | 14 |
| 3 | 14 | 37 | 7 | 58 |
| 4 | 9 | 22 | 3 | 34 |
| Total | 23 | 69 | 14 | 106 |

## Discussion

Here we have documented fisheries mortality in stranded harbor porpoises killed in coastal gill net fisheries in the Mid-Atlantic between 1994 and 1996. However, these deaths represent only an unknown proportion of the total number of porpoises killed in nearshore fisheries along the Mid-Atlantic. Many carcasses were too decomposed to determine cause

of death, and our documentation of entanglement was conservative. In addition, it is likely that not all animals entangled in nearshore gill nets reached the beach.

Detailed examination of stranded carcasses can be a useful means of identifying bycatch of harbor porpoises, and other marine mammals, in nearshore gillnet fisheries. This method is not, however, a surrogate for at-sea observer programs that monitor bycatch rates. The gillnet fisheries that operate along the coastal waters of the Mid-Atlantic are diverse, complex, and their activities poorly documented. An observer program is necessary to provide an unbiased estimate of the number of animals killed in these fisheries, so that a complete assessment can be made of this anthropogenic source of mortality.

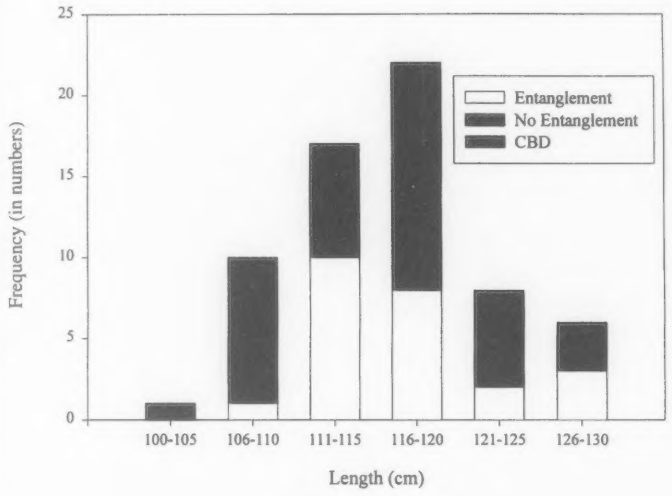


**Figure 4**

Frequency distribution of the lengths of stranded harbor porpoise, *Phocoena phocoena*, carcasses showing evidence of entanglement, no evidence of entanglement, or carcasses for which evidence of entanglement could not be determined owing to advanced decomposition of the carcass.

Accurate assessment of carcasses requires consistent and conservative use of an objective protocol, such as that described by Haley and Read (1993), by trained stranding network personnel. The process of transporting, freezing, and thawing carcasses may obscure subtle evidence of entanglement, so it is important for experienced observers to examine fresh carcasses. At the necropsy workshops, for example, we were unable to assess 14 carcasses that were previously scored as entanglements when examined fresh on the beach.

All stranded porpoises were young and sexually immature. Interestingly, however, both mature and immature porpoises have been taken by gillnet fisheries operating in the Mid-Atlantic. Most of these observed bycatches have been recorded at some distance from shore, suggesting that some age-based segregation occurs in this population during winter. An alternative explanation is that adult porpoises captured in gill nets do not wash ashore. This latter explanation seems unlikely for two reasons. Because of their larger size, carcasses of adults should be more likely to reach shore intact than those of juveniles. In addition, most (17 out of 24) of the observed porpoises taken as bycatch in the Mid-Atlantic were tagged before being discarded; none of these tagged porpoises have subsequently washed up on the beaches. Thus, it appears that only juvenile porpoises are present in the nearshore waters of the Mid-Atlantic during winter.

The proportions of code-2 and code-3 stranded carcasses were not significantly different in the samples of nonentangled and entangled porpoises, indicating that the carcasses of porpoises killed in fisheries were likely originating at similar distances from shore as those animals dying of natural causes. In addition, the lengths of entangled porpoises were similar to those that died from other means. All stranded porpoises, entangled and nonentangled, were from the same segment of the population —young animals in nearshore waters. The distinguishing feature of these two samples is that a larger proportion of the nonentangled animals were emaciated, indicating that they may have died of starvation, whereas most of the entangled animals were in good body condition when they died.

The majority of strandings were reported in Virginia, but this may be due in part to the regular monitoring of beaches in the southern portion of this state. The beaches of Virginia are patrolled frequently by stranding network personnel; therefore stranded porpoises are discovered soon after they appear on the beach. In Maryland beaches are patrolled daily by national and state park personnel. Lower numbers of stranded harbor porpoises in Maryland, therefore, likely reflect lower abundance or different fishing effort and hence fewer strandings in this area. The Outer Banks of North Carolina, by contrast, have not been patrolled frequently, resulting in fewer reports of stranded porpoises. The condition of harbor porpoise carcasses retrieved in North Carolina was poor in relation to those retrieved in

Virginia. Harbor porpoises are small animals and are consumed quickly by scavengers. The National Marine Fisheries Service Beaufort Lab and the University of North Carolina at Wilmington are currently increasing the scope and frequency of beach surveys in the Mid-Atlantic to locate carcasses before they are severely decomposed or scavenged.

We were able to identify the type of net involved in the entanglement of only a small number of animals; therefore at present it is not possible to determine the type of gillnet fisheries in which these porpoises were taken. We assume that all entangled animals were caught in gillnet fisheries operating very close to shore, because the carcasses of porpoises captured farther offshore are not likely to reach the beach intact (see above). Although we could not determine in which fisheries the porpoises were entangled, we can draw attention to patterns of fishing activities that correspond to the time and location of strandings. For example, coastal gillnet fisheries for dogfish (*Squalus* spp.) and American shad (*Alosa sapidissima*) are active during March and April in the Mid-Atlantic, corresponding to the period in which most harbor porpoise strandings occur. A large number of entangled animals stranded in Virginia during the spring of 1994, when the coastal fishery for American shad was operating near the Virginia coast. Records from the state of Virginia indicate that 79 licensed watermen, who individually harvested more than 100 lb of American shad, harvested a total of approximately 387,000 lb of American shad in March and April 1994 (Bower[4]). Both the number of licensed watermen who harvested more than 100 lb of shad and the total harvest decreased dramatically in 1995 and 1996 to 50 and 43 licensed watermen and approximately 161,000 and 235,000 lb, respectively (Bower[4]). We noted a corresponding decrease in the number of stranded harbor porpoises in Virginia in the spring of 1995 and 1996 (Table 1).

Here we have attempted to characterize interactions between nearshore gillnet fisheries and juvenile harbor porpoises in the Mid-Atlantic. The bycatch of harbor porpoises in offshore gillnet fisheries in the Mid-Atlantic is currently monitored closely by a National Marine Fisheries Service observer program and thus can be quantified. Unfortunately, the same is not true for harbor porpoise mortality in the coastal gillnet fisheries. There is a need for a directed observer program for nearshore gillnet fisheries in the Mid-Atlantic to better estimate overall mortality of harbor porpoises owing to fishery interactions in this region. Quantifying this mortality will help in management and conservation efforts for harbor porpoises in the Mid-Atlantic.

[4] Bower, D. Virginia Marine Resources Commission, Newport News, VA 23607. Personal commun.

## Acknowledgments

We would like to thank all the stranding network volunteers in Maryland, Virginia, and North Carolina for their dedicated efforts in collecting harbor porpoise carcasses. We thank Aleta Hohn and two anonymous reviewers for their helpful comments on earlier drafts of this paper. The Smithsonian Institution provided facilities and logistical support for the necropsy workshops. This research was funded by Co-operative Agreements NA57FL0557 and NA67FL0431 from the Northeast Fisheries Science Center to A. J. Read, Purchase Order 43AANF501263 from the Office of Protected Resources, NMFS, to A. J. Read, and Grant N00014-93-1-0640 from the Office of Naval Research to D. A. Pabst.

## Literature cited

**Bravington, M. V., and K. D. Bisack.**
**1996.** Estimates of harbour porpoise bycatch in the Gulf of Maine sink gillnet fishery, 1990–1993. Rep. Int. Whal. Comm. 46:567–574.

**Geraci, J. R., and V. J. Lounsbury.**
**1993.** Marine mammals ashore: a field guide for strandings. Texas A&M University Sea Grant College Program, Galveston, TX, 305 p.

**Haley, N. J., and A. J. Read.**
**1993.** Summary of the workshop on harbor porpoise mortalities and human interactions. NOAA Tech. Memo. NMFS-F/NER-5, 32 p.

**Kastelein, R. A., S. J. van der Sijs, C. Staal, and S. H. Nieuwstraten.**
**1997.** Blubber thickness in harbour porpoises (*Phocoena phocoena*). *In* A. J. Read, P. R. Wiepkema, and P. E. Nachtigall (eds.), The biology of the harbour porpoise, p. 179–199. De Spil Publishers, Woerden, The Netherlands.

**Norris, K. S.**
**1961.** Standardized methods for measuring and recording data on the smaller cetaceans. J. Mamm. 42:471–476.

**Palka, D. L., A. J. Read, A. J. Westgate, and D. W. Johnston.**
**1996.** Summary of current knowledge of harbour porpoises in US and Canadian Atlantic waters. Rep. Int. Whal. Comm. 46:559–565.

**Polachek, T., F. W. Wenzel, and G. Early.**
**1995.** What do stranding data say about harbor porpoises (*Phocoena phocoena*)? Rep. Int. Whal. Comm. (special issue 16):169–179.

**Read, A. J.**
**1994.** Interactions between cetaceans and gillnet and trap fisheries in the Northwest Atlantic. Rep. Int. Whal. Comm. (special issue 15):133–147.

**Read, A. J., and A. A. Hohn.**
**1995.** Life in the fast lane: the life history of harbor porpoises from the Gulf of Maine. Mar. Mamm. Sci. 11:423–440.

**Read, A. J., J. R. Nicolas and J. E. Craddock.**
**1996.** Winter capture of a harbor porpoise in a pelagic drift net off North Carolina. Fish. Bull. 94:381–383.

**Sokal, R. R. and F. J. Rohlf.**
**1981.** Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Company, New York, NY, 859 p.

**Trippel, E. A., J. Y. Wang, M. B. Strong, L. S. Carter and J. D. Conway.**
**1996.** Incidental mortality of harbour porpoise (*Phocoena phocoena*) by the gill-net fishery in the lower Bay of Fundy. Can. J. Fish. Aq. Sci. 53:1294–1300.

# Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters

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**Abstract.**—We examined 1166 black grouper ranging from 155 to 1518 mm TL collected in South Florida waters from 1994 to 1996. Among all black grouper that we sexed, females ranged from 155 to 1310 mm in length (mean= 696, $n$=834), and males ranged from 947 to 1518 mm (mean=1255, $n$=54). Ages of 927 black grouper ranging from 155 to 1518 mm TL were estimated from thin-sectioned otoliths (sagittae). Marginal-increment analysis of grouper 1–7 years old suggested that a single annulus was formed each year during May–June. Black grouper appear to reach a maximum age of at least 33 years, but ages of fish older than 7 years are unvalidated. Growth of the black grouper in our study was rapid until an age of about 10 years and then slowed considerably. The von Bertalanffy growth equation for black grouper was $TL = 1306.2(1\text{-}e^{(-0.169(Age+0.768))})$. Black grouper are protogynous hermaphrodites. We estimated that 50% of the females in the population had reached sexual maturity by 826 mm and an age of 5.2 years. By a length of 1214 mm and an age of 15.5 years, 50% of the females in our sample had transformed into males. The presence of large females in the population suggests that some females may not transform into males. The scarcity of transitional grouper ($n$=1) in our sample suggests that transition occurs quickly. Black grouper appear to spawn yearround, but peak spawning occurs during winter and early spring. Vitellogenic oocytes and oocytes in the final stages of maturation were most common during January–March.

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Fishery Bulletin 96:735–753 (1998).

The black grouper, *Mycteroperca bonaci*, is a large, piscivorous epinepheline serranid that inhabits coral reefs and rocky ledges from North Carolina to Florida, in the Gulf of Mexico, off Bermuda, in the West Indies, and from Central America to southern Brazil (Smith, 1971; Bullock and Smith, 1991). The black grouper supports important commercial and recreational fisheries in South Florida. Commercial landings of the species consistently exceed landings of any other grouper in the Florida Keys. Statewide, the estimated value of Florida black grouper commercial landings has decreased from $2.7 million in 1990 to $1.1 million in 1996 (Florida Department of Environmental Protection Marine Fisheries Information System[1]).

Florida imposes both recreational and commercial regulations on black grouper caught in state waters. There is a recreational minimum size limit of 20 inches (508 mm) total length (TL) and an aggregate daily bag limit of five groupers, including black, yellowfin (*Mycteroperca venenosa*), gag (*M. microlepis*), red (*Epinephelus morio*), yellowmouth (*M. interstitialis*), and scamp (*M. phenax*). The commercial minimum size limit is also 20 inches TL. Federal regulations include a 20-inch minimum size limit and an annual quota for the shallow-water grouper complex, which includes the black grouper. In addition, the activities of commercial longline fishermen east of Cape San Blas, Florida (85°22'W), are restricted to depths greater than 20 fathoms.

Even though the black grouper fishery is regulated in both state and federal waters, little is known about the species' biology, and existing data are inadequate to evaluate the effect of fishing mortality on populations. Like most grouper species, black grouper are protogynous hermaphrodites (García-Cagide and García, 1996). Hermaphroditic species may respond differently to fishing mortality than typical gonochoristic species do, and in some situations, protogynous hermaphrodites may be more sensitive to overfishing than gonochorists (Bannerot et al., 1987). For example, size-selective fishing mortality can greatly skew population sex ratios towards females, which may affect spawning success. In addition, many grouper species form large spawning aggregations that can be easily overfished (Sadovy, 1994). Only Manooch and Mason (1987) have specifically addressed black

[1] Florida Dep. Environmental Protection Marine Fisheries Information System. 1997. Florida Marine Research Inst., Florida Dep. Environmental Protection, 100 Eighth Ave. SE, St. Petersburg, Florida 33701-5095.

grouper life history in South Florida waters, but their study was limited because most of the fish examined were eviscerated and could not be sexed. Consequently, they could not describe sex-specific growth rates, length and age at sexual maturity, length and age at sexual transition, or sex ratios. Furthermore, black grouper often attain much larger sizes than the largest fish aged by Manooch and Mason, who acknowledged that black grouper can probably live longer than the oldest fish they aged (14 years). García-Cagide and García (1996) estimated the length at sexual maturity, the length at transition from female to male, and described seasonal spawning patterns of black grouper captured off Cuba, but they did not estimate ages of the fish they examined. Additional information on growth, maturation, and the length and age at transition are needed to fully assess the status of black grouper stocks. Our article describes growth, length and age at maturity, length and age at sexual transition, dichromatism between the sexes, and seasonal patterns of gonad development. In addition, we used marginal-increment analysis to validate the ages of young black grouper.

## Methods

### Collections

We obtained black grouper from a variety of sources throughout the Florida Keys and Florida's Gulf coast from November 1994 to November 1996. Most of the black grouper in our sample were caught by spear fishermen and landed in the Florida Keys ($n$=733). Commercial hook-and-line and bottom longline black grouper catches were landed and sampled at fish houses in Pinellas County on Florida's Gulf coast ($n$=433). Commercial fishermen captured grouper primarily south of 25°N in depths greater than 37 m, but some catches were made north of 25°N along the Florida Gulf coast to the Florida Panhandle in depths of 37–100 m. Most of the black grouper landed in Pinellas County were caught on or near the Tortugas Banks west of the Florida Keys by commercial longline fishermen, whose operations are restricted by federal regulations to depths greater than 20 fathoms (37 m).

Standard length (SL), fork length (FL), and total length (TL) were measured to the nearest millimeter (mm). Total length was measured following Hubbs and Lagler (1964). Fork length was measured as the center-line length from the tip of the lower jaw to the center of the caudal fin. Unless otherwise indicated, all lengths reported are total lengths. Large grouper were weighed to the nearest 50 grams and smaller grouper were weighed to the nearest gram. Otoliths (sagittae) were removed, rinsed in water, and stored dry until sectioned. Otoliths were weighed to the nearest 0.01 mg. Gonad weight (g) was recorded, and gonad samples for histology were removed from the fish and preserved in 10% buffered formalin; they were later soaked in water for one hour and then stored in 70% ethanol.

We examined most grouper to detect color changes that might be associated with the sex of the fish. All of the fish we examined were dead and had been on ice for several hours to a week. The colors of the pectoral, anal, dorsal, and caudal fins were recorded throughout the year during routine sampling of gonads and otoliths. Only grouper for which we had gonad histology samples were selected for differential color analysis.

### Age and growth

The left sagitta was usually used for age estimation; however, in cases where the left otolith was broken, lost, or damaged during processing, the right otolith was substituted. We prepared most otoliths by using a Buehler Isomet low-speed saw with a diamond blade to cut three or four thin sections to a thickness of approximately 0.5 mm, one of which was through the otolith core. Sections were then mounted on a microscope slide with Histomount. Initially, we prepared some otoliths for age estimation by embedding them in Spurr, a high-density plastic medium (Secor et al., 1992). A 1- to 2-mm-thick transverse section containing the otolith core was cut. The section was mounted on a microscope slide with thermoplastic glue (CrystalBond 509 adhesive) and polished with "wet/dry" sandpaper (grit sizes ranging from 220 to 2,000) until annuli were visible. Sections were then polished on a Buehler polishing cloth with 0.05-μ gamma alumina powder to remove scratches. There was no consistent difference in the quality of either preparation technique. Mounting the sections in Histomount took less time than embedding them in Spurr, so we adopted it as our standard protocol.

Annuli were counted using compound microscopes equipped with transmitted light and dissection microscopes equipped with reflected light. Two independent readers counted annuli on each otolith section three times each, for a total of six counts for each otolith. Thirty-two otoliths were considered unreadable by one or both readers and were discarded from our analysis before the completion of six readings. Annulus counts for individual otoliths often varied among readings. We established criteria for accepting or rejecting individual otoliths by calculating a coefficient of variation:

$$CV = (SD/\overline{X} \times 100),$$

where $SD$ = the standard deviation of counts for a given otolith; and
$\overline{X}$ = the mean annulus count for a given otolith.

After six readings were completed for all otoliths, otoliths for which the CV>12% were again read independently by each reader without knowledge of previous annulus counts. The count with the greatest difference from the mean of all counts was then discarded and replaced with a new count made by the reader whose reading was discarded. This protocol was repeated twice, and if the CV for a given otolith remained >12%, the otolith was rejected from our analysis.

The von Bertalanffy (1957) growth equation $TL_t = L_\infty(1-e^{(-K(t-t_0))})$ was fitted to observed age-length data with nonlinear regression procedures. Age was estimated as the mean of all six annulus counts. We did not attempt to backcalculate the length at which the last annulus was formed because of the variability among annulus counts for most otoliths. Our estimates of length at age thus include some seasonal growth that occurred after the formation of the final annulus. To estimate mean length at age, we rounded all ages to the nearest integer, but in growth and maturity models the mean age was not rounded to an integer and fractional ages were used. Length-weight regressions were calculated by linear regression of $\log_{10}$-transformed data.

## Age validation

Measurements for marginal-increment analysis were made with a digital image-processing system on an axis extending along the sulcal ridge from the otolith's core to the proximal margin of the section. Because many black grouper otoliths were difficult to read, we selected only otoliths for which there was 100% agreement among all readings for marginal-increment analysis. In addition, we restricted measurements to otoliths with from one to seven annuli because it was difficult to measure the more closely spaced outer annuli of older fish. We expressed the distance from the final annulus to the otolith's edge (marginal increment) as a percentage of the distance between the last two annuli formed on the otolith. For all grouper, the distance between the otolith core and the first annulus ($r_1$) was typically much greater than the distance between the first and second annuli ($r_2$–$r_1$). For this reason, we divided the distance between the first and second annuli by the distance between the otolith's core and the first annulus for each otolith measured and then calculated the mean of this number for the entire sample:

$$\frac{\sum_{i=1}^{n}((r_2 - r_1)/r_1)_i}{n} = 0.353. \qquad (SE = 0.0065)$$

We then estimated the expected distance between the first and second annulus for each age-1 black grouper otolith as a function of the distance between the otolith's core and the first annulus. The percent marginal increment for age-1 fish was then calculated as

$$(MI/(0.353 \times r_1)) \times 100,$$

where $MI$ = the marginal increment.

We then plotted the percent marginal increments as a function of capture month for 1995–96, the period during which we made regular monthly collections.

## Reproduction

Histological sections of gonads from 857 black grouper that ranged in length from 155 to 1518 mm were prepared and assessed for reproductive state. Gonad samples were processed histologically with `,Modification of the periodic acid Schiff's (PAS) stain for glycol-methacrylate sections, with Weigert's iron-hematoxylin as a nuclear stain, and with metanil yellow as a counterstain (Quintero-Hunter et al., 1991).

Oocytes were staged and counted from histological preparations at 100× with a compound microscope attached to a digital image-processing system. Four oocyte stages were recognized in black grouper ovaries: primary growth, cortical alveolar, vitellogenic, and oocytes in the final stages of maturation (Wallace and Selman, 1981). The final stages of oocyte maturation included yolk coalescence, germinal vesicle migration, germinal vesicle breakdown, and hydration. At least 300 oocytes per slide were staged and counted in arbitrarily chosen fields, and frequencies were expressed as a percentage of the total count. We counted all oocytes that had at least 50% of their area visible in a field before moving to the next field.

Gonads were classified on the basis of the maturity criteria of Moe (1969). Female grouper were considered sexually mature if ovaries contained vitellogenic oocytes or contained evidence of widespread atresia consistent with gonadal recrudescence. Mature females included Moe's classes 2, 3, and 4.

Monthly median gonadosomatic indices (GSI) were plotted to show seasonal reproductive patterns. Gonadosomatic indices were calculated for 201 sexu-

ally mature female grouper ranging in length from 565 to 1310 mm and for 43 sexually mature male grouper ranging in length from 947 to 1518 mm as

$$GSI = (GW / (TW - GW)) \times 100,$$

where $GW$ = total gonad weight (g); and
$TW$ = total fish weight (g).

We measured the diameters of whole oocytes from six females whose ovaries contained numerous hydrated oocytes. Samples of ovarian tissue that were preserved in 10% formalin were rinsed briefly in water and transferred to 33% glycerin for measurements. Diameters of at least 300 oocytes for each female were measured with a digital image-prossessing system. Only oocytes larger than 0.2 mm in diameter were measured.

We estimated the length at which 50% of the females in the population reached sexual maturity by fitting a logistic function to the percentage of females that were sexually mature and their respective lengths. A similar function was fitted to the percentage of the population made up by females to estimate the length at which 50% of the females in the population transformed into males. The parameter $b$ in Table 1 is the inflection point of the logistic curves and is the estimate of the length or age at which 50% of the females in the population were sexually mature or the length or age at which 50% of the population had undergone transition from female to male. Sometimes distinguishing between the gonads of sexually immature grouper and the regressed gonads of mature fish was difficult, and we eliminated 29 females that ranged in length from 586 to 894 mm from our analysis of maturation because we were uncertain of their maturity. Hunter et al. (1992) recommended that only fish collected early in the spawning season be used to estimate the length at 50% maturity, but because we found evidence of some spawning during most of the year, we included all months in our analysis.

## Results

The 1166 black grouper we examined ranged from 155 to 1518 mm in length. Among black grouper that we sexed, females ranged from 155 to 1310 mm in length (mean=696.3, SD=204.53, $n$=834) and males ranged from 947 to 1518 mm (mean=1254.9, SD=126.19, $n$=54; Fig. 1). Our sample contained 276 grouper that were not sexed, usually because the grouper had been eviscerated at sea by fishermen. The sex ratio of our sample was 1:15.4 (male:female); greatly skewed towards females. Black grouper landed in Pinellas County on Florida's Gulf coast by commercial fishermen were much larger than those landed in the Florida Keys (Fig. 2). The male:female sex ratio of the black grouper we sampled that were landed in the Florida Keys was 1:58.7, and the sex

**Table 1**

The relations between standard length, fork length, and total length, between length and weight, between the percentage of females that were sexually mature and length, and between the percentage of the population made up by females and length of black grouper, *Mycteroperca bonaci*, from South Florida waters. TL = total length (mm), FL = fork length (mm), SL = standard length (mm), WT = whole weight (g), GWT = gutted weight (g), OTOWT = otolith weight (g), and AGE = age (years). The total length range for all length-length regressions was 238–1518 mm and for the length-weight regression was 177–1518 mm; the total weight range for the total weight-gutted weight regression was 467–61,462 g; the age range for the age-otolith weight regression was 1–30.5 years.

| | | | $Y = a+bX$ | | |
|---|---|---|---|---|---|
| $Y$ | $X$ | $n$ | $a$ (1 SE) | $b$ (1SE) | $r^2$ |
| SL | FL | 1,134 | –23.712 (0.6852) | 0.883 (0.0008) | 0.999 |
| SL | TL | 1,141 | –21.611 (0.7743) | 0.858 (0.0009) | 0.999 |
| FL | SL | 1,134 | 27.607 (0.7514) | 1.131 (0.0011) | 0.999 |
| FL | TL | 1,150 | 1.641 (0.5223) | 0.973 (0.0006) | 0.999 |
| TL | SL | 1,141 | 26.186 (0.8757) | 1.164 (0.0012) | 0.999 |
| TL | FL | 1,150 | –1.317 (0.5378) | 1.028 (0.0006) | 0.999 |
| WT | GWT | 638 | 81.519 (12.9253) | 1.056 (0.0014) | 0.999 |
| $\log_{10}$WT | $\log_{10}$TL | 772 | –5.457 (0.0323) | 3.218 (0.0115) | 0.995 |
| | | | $Y = a+b\times\sqrt{X}$ | | |
| OTOWT | AGE | 389 | –0.1026 (0.0036) | 0.1210 (0.0014) | 0.952 |
| | | | $Y = \left(1/\left(1+e^{(a(X-b))}\right)\right)\times 100$ | | |
| % Mature (Females) | TL | 782 | –0.0194 (0.0017) | 826.0 (4.75) | 0.592 |
| % Mature (Females) | AGE | 617 | –1.375 (0.1341) | 5.20 (0.078) | 0.555 |
| % Females | TL | 888 | 0.016 (0.0013) | 1214.4 (5.05) | 0.618 |
| % Females | AGE | 694 | 0.355 (0.0287) | 15.55 (0.382) | 0.551 |

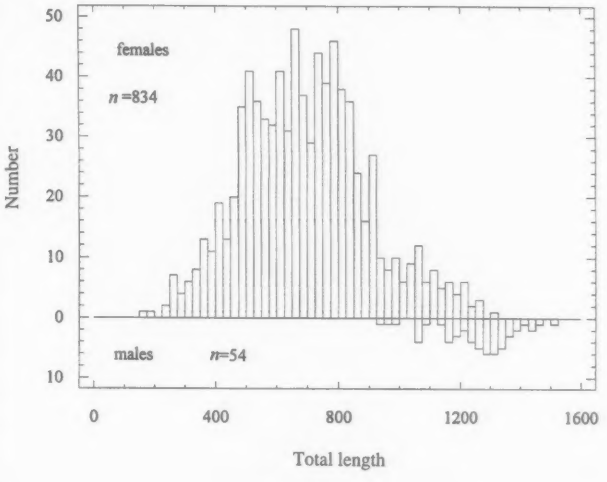


**Figure 1**

Total lengths (mm) of male and female black grouper, *Mycteroperca bonaci*, sampled from South Florida waters during 1994–96.

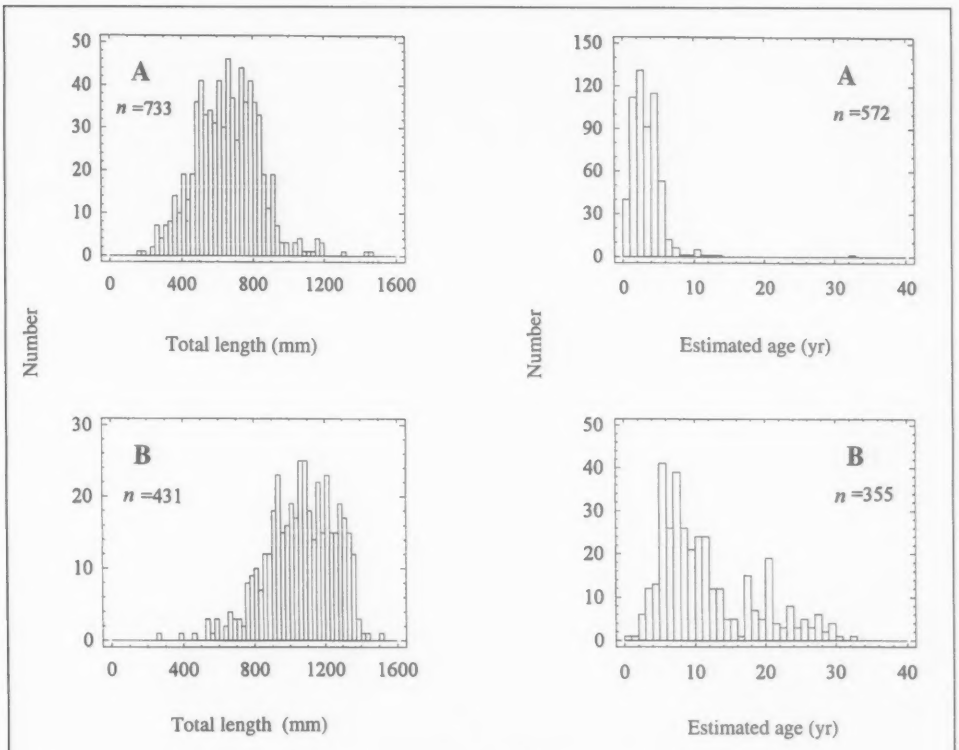


**Figure 2**

Total lengths (mm) and ages (years) of black grouper, *Mycteroperca bonaci*, landed in the Florida Keys (**A**) and in Pinellas County on Florida's Gulf coast (**B**).

ratio of black grouper landed in Pinellas County by commercial fishermen was 1:3.1. The pooled length-weight equation for sexed and unsexed fish and the relations between SL, FL, and TL are presented in Table 1.

## Age and growth

When viewed with reflected light, black grouper otoliths have narrow, opaque (bright) annuli that alternate with broad translucent (dark) zones (Fig. 3, A and B). Proceeding from the otolith's core towards the otolith's proximal margin, these translucent zones become increasingly opaque in appearance as the otolith grows. In the outer portion of the otoliths from older grouper (>10 years old), the dark translucent zones are narrow. In some individuals, the annuli are indistinct and irregular in appearance, which makes age estimation difficult. In older grouper, the annuli become closely spaced near the edge of the otolith, and this also makes age estimation difficult (Fig. 3C). Often otoliths of older grouper (>15 years) were more easily read under transmitted light and compound microscopes; otoliths from younger fish were often more easily read under reflected light. Overall, the otoliths from black grouper are similar in appearance to those of other grouper species we have examined in our laboratory.

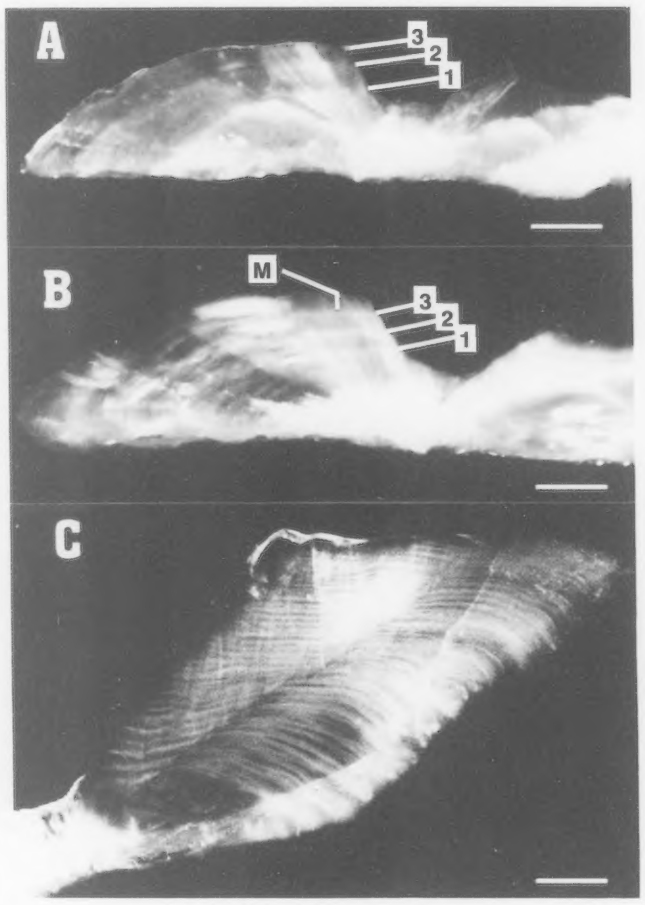


**Figure 3**

(**A**) Sectioned sagitta from a 3-year-old black grouper, *Mycteroperca bonaci* (508 mm TL), captured in May 1996. The third annulus is on the edge of the otolith. (**B**) Sectioned sagitta from a 3+ year-old black grouper (671 mm TL) captured in January 1996. Note the wide marginal increment (m) subsequent to the third annulus. (**C**) Sectioned sagitta from a 33-year-old male black grouper (1427 mm TL) captured in June 1996. Note the close spacing of annuli that caused difficulty in age estimation. Scale bars = 500 microns.

Marginal-increment analysis of otoliths from grouper 1–7 years old suggested that one annulus had been formed during April–June each year (Fig. 4). Median marginal increments had a consistent seasonal minimum during May–July and a maximum during December–March in both 1995 and 1996. Marginal increments during April–June tended to be either large (>75%) or small (<25%), suggesting that most individuals either had wide margins, as expected just prior to annulus formation, or had just formed an annulus and had either no marginal increment or a narrow margin. By June or July, individuals with wide margins were no longer present, suggesting that annulus formation was completed.

Of 1059 otoliths that we examined, 132 (12.5%) were rejected because of disagreements among readings. We had total agreement among annulus counts for 403 otoliths, but all of these otoliths were from grouper estimated to be less than 10 years old. The length-frequency distribution of fish whose otoliths were rejected because they were unsuitable for age estimation was not significantly different from that of fish whose otoliths were readable (Kolmogorov-Smirnov two-sample test, two-sided test statistic = 1.319, $P$=0.062), but the otolith weight distribution of fish whose otoliths were unreadable was significantly different from that of fish whose otoliths were readable (Kolmogorov-Smirnov two-sample test, two-sided test statistic=2.017, $P$<0.001). Otolith weight

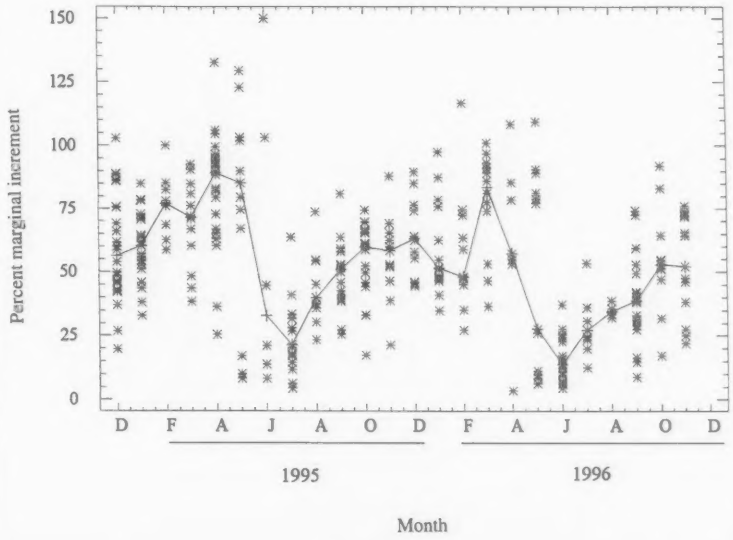


**Figure 4**

Percent marginal increments (*) and medians (+ and line) for black grouper, *Mycteroperca bonaci*, ages 1–7 captured from South Florida waters ($n$=330).

was closely related to fish age ($r^2$=0.952, Table 1, Fig. 5A), and the median weight (0.2437 g) of otoliths rejected as unreadable was significantly greater than that of otoliths that were readable (0.1571 g; Mann-Whitney $W$ test; $W$=12,492.0, $P$<0.001).

Black grouper growth was rapid until an age of about 10 years and then slowed considerably (Table 2, Fig. 5B). Most of the fish in our sample were from 2 to 10 years old, and the most abundant age classes were 2–6 years old (Fig. 6). The two oldest black grouper examined were a 33-year-old (1325-mm) fish that was not sexed and a 33-year-old (1427-mm) male (Table 2). The oldest female was a 1275-mm fish estimated to be 23 years old (Fig. 6); the largest female was 1310 mm long (Fig. 1) and was estimated to be 12 years old. Only three female black grouper were estimated to be over 20 years old. The smallest and youngest male was a 947-mm fish estimated to be 6 years old (Fig. 1 and 6). Black grouper landed in Pinellas County on Florida's Gulf coast by commercial fishermen were older overall than those landed in the Florida Keys, where only one fish older than 15 years was examined (Fig. 2). Estimates of von Bertalanffy growth model parameters are presented in Table 3.

## Sexual maturation and transition

Black grouper, like most epinepheline serranids, are protogynous hermaphrodites. Protogyny was suggested by the presence of peripheral sperm-collecting sinuses rather than the centrally located sinuses typical of gonochorists. Furthermore, a membrane-lined central lumen that was not used for sperm transport was present in testes and was a structural remnant of the ovarian lumen (Sadovy and Shapiro, 1987). Additional evidence of hermaphroditism was the presence of atretic vitellogenic oocytes in a transitional gonad containing proliferating testicular tissue (Fig. 7A). A second grouper that we would have considered transitional except for the presence of some mature sperm in peripheral sperm sinuses also contained degenerating vitellogenic oocytes (Fig. 7, B and C); this fish was considered to be a functional male. Transitional grouper also contained numerous PAS-positive melanomacrophage centers (Ravaglia and Maggese, 1995) referred to as "yellow-brown bodies" by Sadovy and Shapiro (1987). When stained with the PAS stain, these structures are brilliant purple. Melanomacrophage centers are thought to be active in degrading atretic oocytes, postovulatory follicles, and residual cells of the spermatogenic cycle (Chan et al., 1967; Ravaglia and Maggese, 1995). Many mature males contained scattered primary growth stage oocytes throughout the testis. We also noted that small amounts of testicular tissue were often present in functional ovaries that showed no evidence of undergoing transition. Finally, the differences in male and female length-frequency distri-

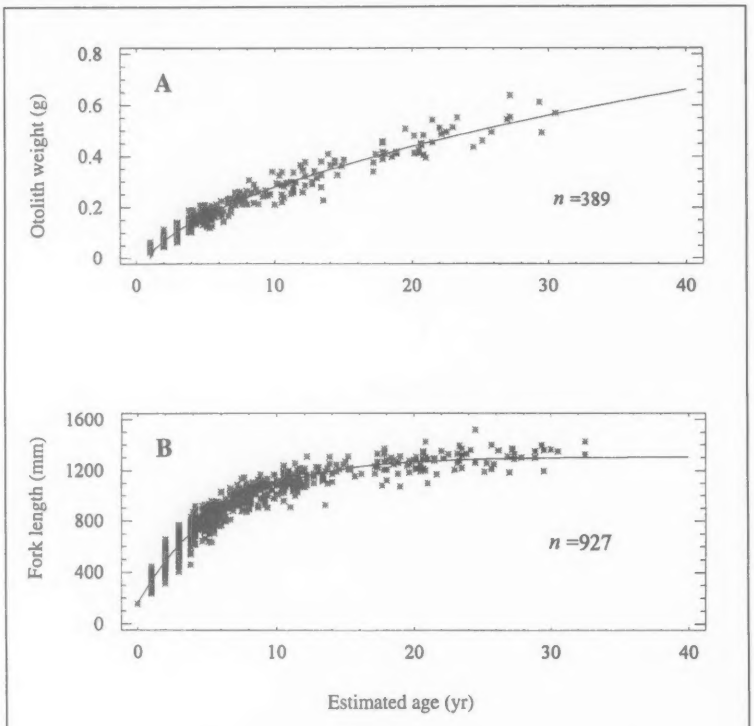


**Figure 5**

(**A**) The otolith weight–age relation for black grouper *Mycteroperca bonaci*, collected from South Florida waters. The equation for the relation is presented in Table 1. (**B**) Observed and predicted total lengths (mm) from the von Bertalanffy growth model for sexed and unsexed black grouper, *Mycteroperca bonaci*.

butions and the absence of small or young males are consistent with our hypothesis of protogynous hermaphroditism (Fig. 1).

We estimated that 50% of the females in the population reached sexual maturity by 826 mm and an age of 5.2 years (Table 1, Fig. 8). The smallest sexually mature female in our sample was 508 mm, and the youngest sexually mature female was 2 years old. All of the ovaries we examined contained primary growth stage oocytes. Cortical alveolar stage oocytes occurred only in ovaries from grouper larger than about 500 mm and older than 2 years, and they were common only among grouper larger than about 600 mm and older than 3 years (Fig. 9A). Vitellogenic oocytes were found only in ovaries from fish larger than about 600 mm and older than 2 years and were common only among grouper larger than 800 mm and older than 5 years (Fig. 9B). The length and age at which vitellogenic oocytes were commonly found agrees well with our estimate of the length and age at which 50% maturity was reached, suggesting that misclassification of regressed gonads did not greatly bias our estimates.

Transition from female to male was also partially a function of length and age. By a length of 1214 mm and an age of 15.5 years, 50% of the females in the population had transformed into males (Table 1, Fig. 8). We found only one transitional fish (1030 mm long; Fig. 7A) and a second grouper (947 mm long) that appeared to have recently completed transition (Fig. 7, B and C). We did not find any immature males (Moe's class 6) in our sample, suggesting that males become sexually active soon after transition. Most (91%) of the males we examined had ripe testes (Moe's class 9) that contained mature sperm.

Fin pigmentation differed between sexes in black grouper (Fig. 10). Ontogenetic color change independent of sex was ruled out because relatively small males (<1000 mm) displayed the male color phase, but the oldest and largest females (e.g., 1255 mm) did not. The pectoral fins, anal fin, and caudal fin were dark colored in females, but these fins were jet

black in males (Fig. 10, A–C). The jet black pigmentation of the pectoral fin was most apparent on the medial side. The dorsal interspinous membrane of females was yellow to dusky (Fig. 10D). In males, this membrane was usually dark black but was occasionally yellow or yellowish with black tips. Because this membrane can be yellow in both males and females, the coloration of this fin was the least reliable indicator of sex. The distal one-third of the soft dorsal fin was dark black in both sexes and was not useful in determining sex.

## Seasonality of gonad development

The frequencies of the four oocyte stages we counted in black grouper ovaries had a seasonal pattern that was repeated in both 1995 and 1996 (Fig. 11, A and B). Vitellogenic oocytes were present in greatest number in January–March in both 1995 and 1996 and were present in all months except September–November 1996. Cortical alveolar stage oocytes were also present in all months except September–November 1996 and were most abundant during December–April. Primary growth stage oocytes were present during all months and made up at least 55% of the total number of oocytes present. Oocytes in the final stages of maturation were not abundant, but they were most common during December–May (Fig. 11B). We examined six females with hydrated ovaries: one was caught during October, one in December, and four in March. The ovaries from these grouper contained a group of vitellogenic oocytes that ranged from 0.5 to 0.7 mm in diameter and a clutch of hydrated oocytes that ranged from 0.8 to 1.2 mm in diameter (Fig. 12). Most of the oocytes <0.2 mm in diameter, which we did not measure, were primary growth stage or cortical alveolar stage oocytes. Postovulatory follicles were not recognized in any female examined.

Females with the greatest GSIs (>5) were captured during February–March (Fig. 13). Male GSIs (range 0.086–0.399, $n$=43) were generally much less than female GSIs (range 0.032–10.108, $n$=201) and had no

**Table 2**

Average observed and predicted total lengths (mm) for sexed and unsexed black grouper, *Mycteroperca bonaci*, and average observed lengths for females and males. The average observed length at age includes some seasonal growth that occurred after the formation of the final annulus. Values in parentheses are standard error and sample size.

| | Sexed and unsexed | | Females | Males |
|---|---|---|---|---|
| Age (yr) | Average observed | Predicted | Average observed | Average observed |
| 0 | 155 (1) | 159 | 155 (1) | |
| 1 | 338 (8.7;41) | 337 | 339 (8.9; 39) | |
| 2 | 486 (5.7;113) | 487 | 486 (5.7;112) | |
| 3 | 606 (5.7;136) | 614 | 607 (5.7;134) | |
| 4 | 733 (5.5;155) | 722 | 734 (5.7;147) | |
| 5 | 814 (5.8;113) | 813 | 814 (6.1;97) | |
| 6 | 871 (7.7;70) | 889 | 857 (10.2;41) | 947 (1) |
| 7 | 972 (9.1;40) | 954 | 966 (13.6;20) | 1062 (1) |
| 8 | 1008 (11.6;39) | 1009 | 998 (20.5;15) | 1006 (46.0;2) |
| 9 | 1038 (12.3;31) | 1055 | 1024 (19.8;14) | 1058 (1) |
| 10 | 1074 (12.3;16) | 1094 | 1078 (14.6;5) | 1167 (1) |
| 11 | 1116 (13.1;33) | 1127 | 1147 (27.0;10) | 1130 (22.8;5) |
| 12 | 1138 (14.4;21) | 1155 | 1131 (29.0;9) | |
| 13 | 1164 (11.6;11) | 1178 | 1182 (16.0;6) | 1172 (1) |
| 14 | 1175 (30.6;11) | 1198 | 1192 (18.5;2) | 1222 (30.5;2) |
| 15 | 1239 (14.4;7) | 1215 | 1213 (1) | 1266 (26.0;3) |
| 16 | 1165 (10.5;2) | 1229 | | |
| 17 | 1244 (21.2;6) | 1241 | 1270 (1) | 1283 (47.5;2) |
| 18 | 1252 (30.3;10) | 1251 | | |
| 19 | 1226 (35.5;6) | 1260 | | 1285 (1) |
| 20 | 1248 (12.9;9) | 1267 | 1220 (1) | |
| 21 | 1264 (20.2;15) | 1273 | 1195 (1) | 1315 (38.7;4) |
| 22 | 1278 (29.6;5) | 1278 | | |
| 23 | 1298 (27.3;6) | 1283 | 1275 (1) | 1326 (68.5;2) |
| 24 | 1320 (26.6;5) | 1286 | | 1370 (1) |
| 25 | 1307 (72.2;4) | 1289 | | 1390 (128.0;2) |
| 26 | 1305 (44.5;4) | 1292 | | |
| 27 | 1309 (23.3;7) | 1294 | | 1291 (38.3;4) |
| 28 | 1300 (1) | 1296 | | |
| 29 | 1349 (28.3;3) | 1298 | | 1349 (28.3;3) |
| 30 | 1307 (55.9;3) | 1299 | | |
| 31 | 1350 (1) | 1300 | | 1350 (1) |
| 32 | 1301 | | | |
| 33 | 1376 (51.0;2) | 1302 | | 1427 (1) |

**Table 3**

Parameter estimates for the von Bertalanffy growth model for black grouper, *Mycteroperca bonaci*, collected from the waters of South Florida. Values in parentheses are standard errors.

| $n$ | $L_\infty$ (mm) | $K$ | $t_0$ | adjusted $r^2$ |
|---|---|---|---|---|
| 927 | 1306.2 (8.05) | 0.169 (0.0037) | –0.768 (0.0640) | 0.941 |

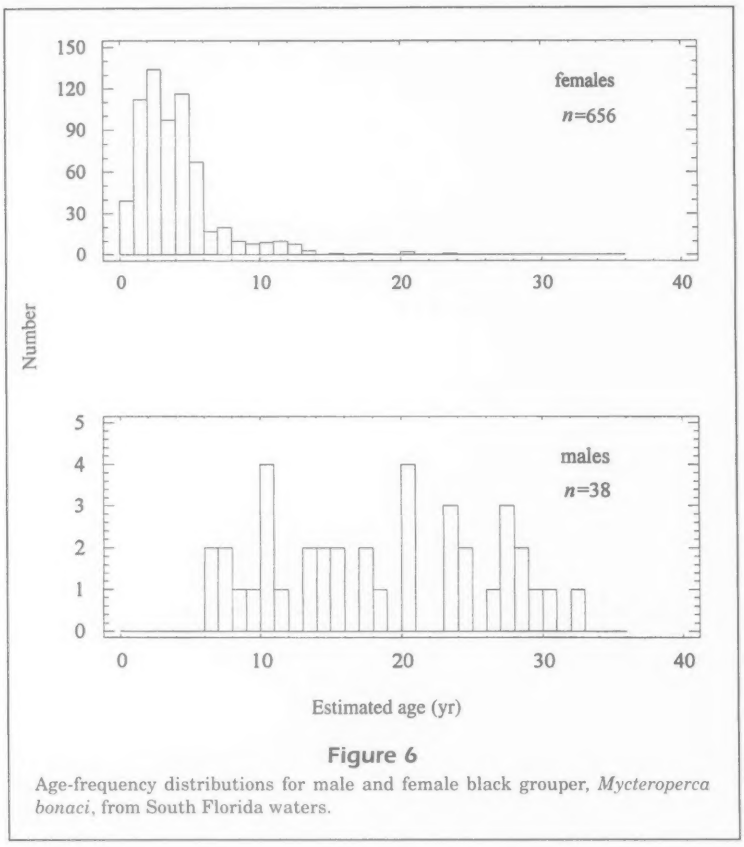


**Figure 6**

Age-frequency distributions for male and female black grouper, *Mycteroperca bonaci*, from South Florida waters.

seasonal trend (Fig. 13). Testes from sexually mature males ranged in weight from 14 to 197 g and were much smaller overall than ovaries from sexually mature females, which ranged in weight from 1 to 1354 g.

## Discussion

We obtained black grouper from a variety of fishery-dependent and fishery-independent sources, and it is difficult to assess the extent to which the length and age structure of our sample reflects that of the population or the degree to which different fishing gears biased the various samples. Although we sampled grouper that were landed in two geographically separate locations, the Florida Keys and in Pinellas County on Florida's Gulf coast, most of the grouper landed in both locations were caught in the waters off the Florida Keys. Most of the large (>1000 mm) fish and most of the males that we examined came from commercial fish houses on Florida's Gulf coast, and most of these grouper were caught on the Tortugas Banks west of the Florida Keys. Fish obtained from Keys headboats, fish houses, and spearfishermen were smaller and younger overall than grouper sampled from fish houses in Pinellas County (Fig. 2). Most black grouper landed in Pinellas County fish houses were caught with commercial longlines in depths greater than 37 m (20 fathoms); most of the fish landed in the Keys were captured by spear fishermen, principally in water 6–50 m deep. The greater depths fished and the greater distances traveled by Gulf-coast commercial fishermen to presumably more remote fishing areas probably account for the differences between the two samples.

### Age and growth

Black grouper appear, on the basis of marginal-increment validation for grouper 1–7 years old, to form a single annulus each year in late spring or early summer. We were unable to validate that annuli are annual marks in older grouper, and the accuracy of our age estimates for older fish is unknown. The annuli we counted on otolith sections from older grouper were similar in appearance to validated annuli;

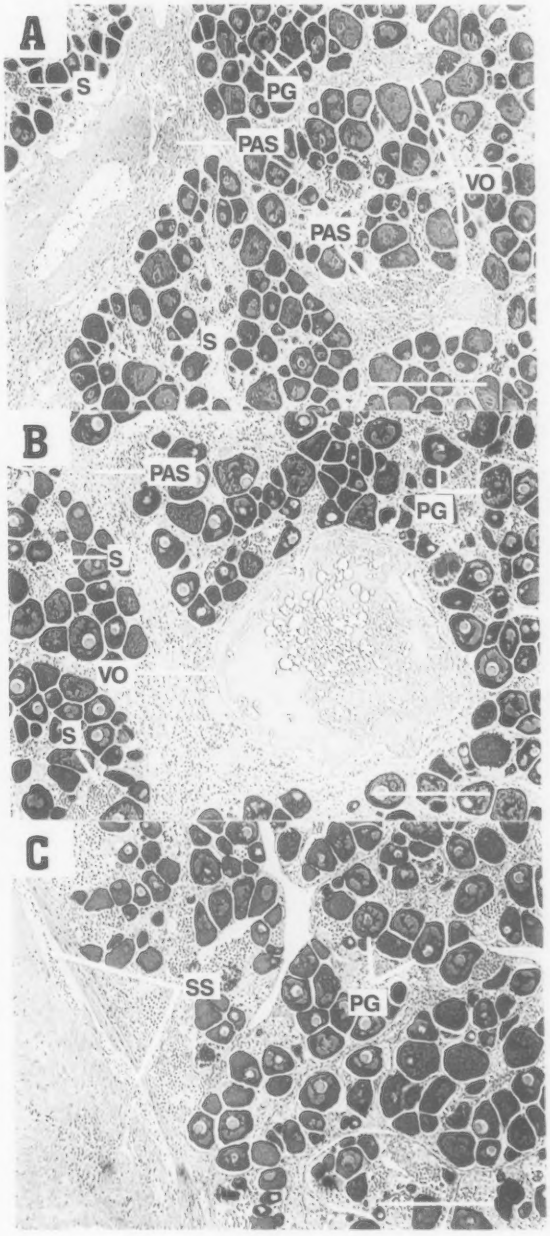


**Figure 7**

(**A**) A transitional gonad from a 1030-mm-TL black grouper, *Mycteroperca bonaci*, captured in September 1994 with proliferating spermatogenic tissue in sperm cysts (S), primary growth stage oocytes (PG), degenerating vitellogenic oocytes (VO), and PAS-positive melanomacrophage centers (PAS). (**B**) A 947-mm-TL black grouper captured in November 1995 with proliferating spermatogenic tissue in sperm cysts (S), primary growth stage oocytes (PG), degenerating vitellogenic oocytes (VO), and PAS-positive melanomacrophage centers (PAS). (**C**) The same ovary as in B showing the presence of mature sperm in peripheral sperm sinuses (SS). Scale bars = 200 microns.

however, as annuli became progressively more closely spaced, age estimation became more difficult. Additional work is needed to assess the accuracy of our age estimates for older grouper; consequently, our age estimates predicted lengths at age, and growth model parameters should be used with caution. From their analyses of marginal increments, Manooch and Mason (1987) also reported that black grouper from the Florida Keys form a single annulus each year. They found that annuli were usually formed during March–May, about a month earlier than we estimated. This difference is probably a result of differences in interpretation of the appearance of an annulus present on the otolith margin. Other congeners also form annuli during late spring and early summer. Collins et al. (1987) reported that gag, *M. microlepis*, off the southeastern U.S. coast form annuli during late spring to late summer, and Hood and Schlieder (1992) suggested that gag form annuli during summer in the eastern Gulf of Mexico. Bullock and Murphy (1994) reported that yellowmouth grouper, *M. interstitialis*, form opaque bands during late summer and early fall. Matheson et al. (1986) found that scamp, *M. phenax*, in North Carolina waters form an annual mark during April or May.

We rejected 12.5% of the otoliths we sectioned as unreadable, and this is a possible source of bias to our growth model parameters. Otolith weight is a useful predictor of age of black grouper ($r^2$=0.952), and the otoliths that we rejected were generally heavier and thus probably older than most of those that we considered readable. In addition, although the length-frequency distribution of fish whose otoliths were rejected was not significantly different from that of all fish whose otoliths were readable, the significance level of the test ($P$=0.062) was close enough to 0.05 to cause us to suspect that the distributions could have been different. If so, we may have rejected as unreadable more otoliths from large grouper than from small grouper. We may also have tended to exclude slower-growing older grouper from our sample of aged fish, and this could have biased our growth models. However, when we recalculated growth models and included all fish regardless of CV, the resulting growth-parameter estimates were all within one standard error of those in Table 3. Thus any bias to the growth model caused by our rejection of otoliths from older grouper appears to be negligible. Our choice of a threshold CV of 12% was arbitrary, but growth model parameters did not appear to be sensitive to the choice of CV thresh-

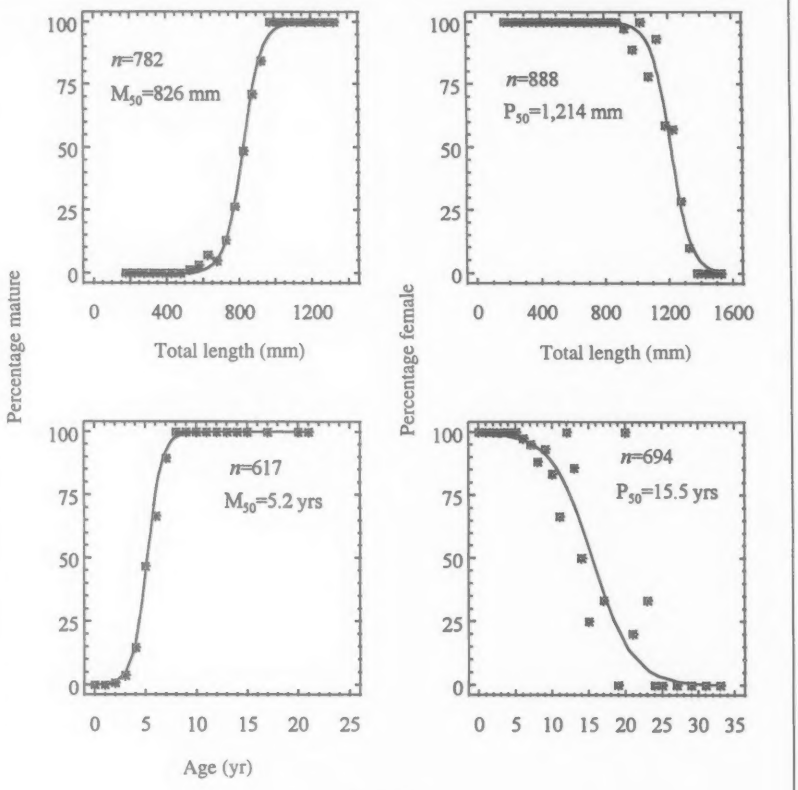


**Figure 8**

Logistic curves describing the percentage of female black grouper, *Mycteroperca bonaci*, collected from South Florida waters that were sexually mature and the percentage of our black grouper Sample that was made up by females as a function of both total length (mm) and age (years). $M_{50}$ is the length or age predicted by the logistic regression at which 50% of the female black grouper were sexually mature, and $P_{50}$ is the length or age predicted by the logistic regression at which 50% of the black grouper in our sample were females.

olds. When we recalculated growth models with CV thresholds of 10% and 5%, our estimates of $L_\infty$ and $K$ remained within two standard errors of the estimates at a CV of 12%, but the numbers of otoliths eliminated from our analysis increased substantially at lower CV thresholds.

The two oldest black grouper in our sample were both estimated to be 33 years old, considerably older than Manooch and Mason's (1987) oldest grouper (14 years). Manooch and Mason sampled hook-and-line-caught black grouper from Keys headboats during 1978–85 and speculated that the maximum age attained by black grouper was probably about 19 years. The presence of older fish in our sample is probably because our sample contained black grouper that were larger (largest 1518 mm) than those examined by Manooch and Mason (largest aged 1180 mm; largest measured 1259 mm). We measured 125 black grouper longer than 1200 mm and aged 94 of these. Of the 98 grouper that we estimated to be older than 14 years, 84% were longer than 1200 mm and were longer than any fish aged by Manooch and Mason. Most of the large black grouper in our sample were caught by commercial longliners and landed in Pinellas County; the lengths and ages of the grouper in our sample that were landed in the Keys were similar to those of Manooch and Mason (1987).

Our estimates of length at age are similar to those of Manooch and Mason (1987), and the predicted growth curves from both data sets are in general agreement (Fig. 14). In some cases, such as age classes 5–9, our estimates of mean length at age are

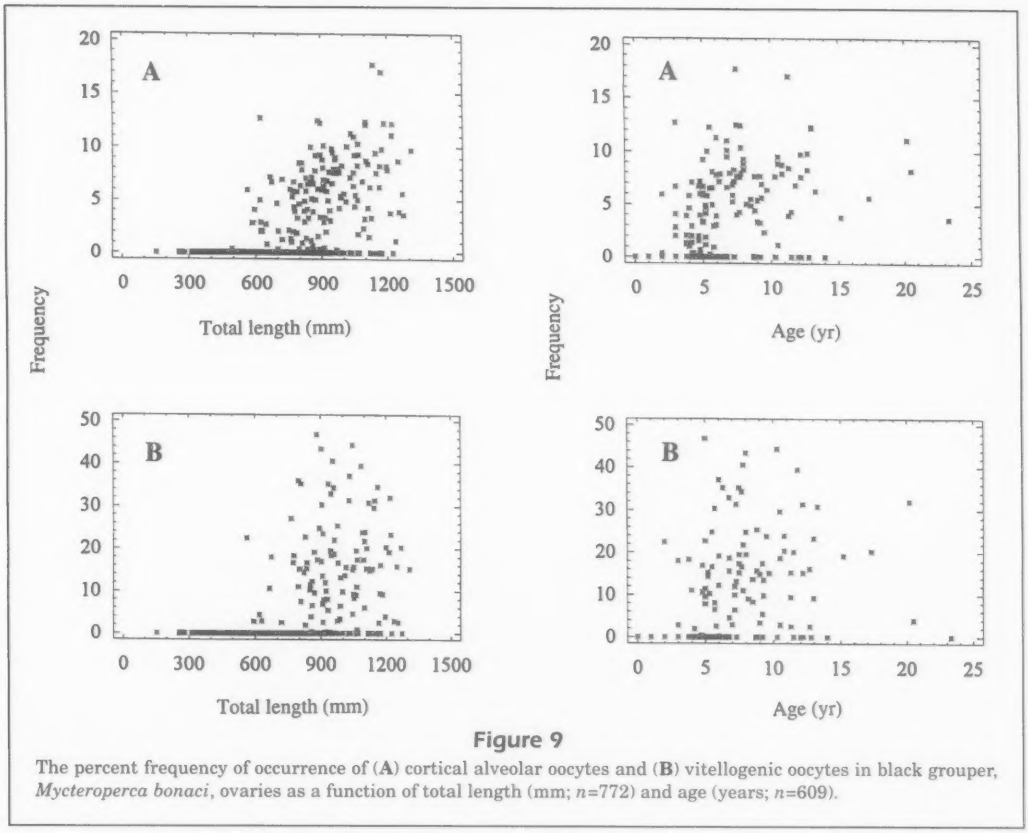


**Figure 9**

The percent frequency of occurrence of (**A**) cortical alveolar oocytes and (**B**) vitellogenic oocytes in black grouper, *Mycteroperca bonaci*, ovaries as a function of total length (mm; $n$=772) and age (years; $n$=609).

greater than those of Manooch and Mason, but their estimates for these age classes fall below not only our predicted lengths but also their predicted lengths. The von Bertalanffy growth model parameters estimated by Manooch and Mason were $L_\infty$ = 1,352 mm, greater than our estimate of $L_\infty$ = 1,306 mm, and $K$ = 0.1156, less than our estimate of $K$ = 0.169.

Black grouper, though not as large as jewfish or warsaw grouper, are among the largest species of grouper, and it is not surprising that they attain ages over 30 years. Estimates of the longevity of other grouper species are similar to our estimates for black grouper. Hood and Schlieder (1992) and Collins et al. (1987) estimated a maximum age of 21–22 years for gag, Bullock et al. (1992) estimated a maximum age of 37 years for jewfish (*E. itajara*), Bullock and Murphy (1994) estimated a maximum age of 28 years for yellowmouth grouper, Manooch and Mason (1987) reported that warsaw grouper (*E. nigritus*) reach 41 years, and Moe (1969) reported a red grouper (*E. morio*) 25 years old. The growth rate ($K$=0.169/year) of black grouper is similar to that estimated for gag ($K$=0.166/year, Hood and Schlieder, 1992), but is greater than the growth rates estimated for many other grouper species: jewfish $K$ = 0.126/year (Bullock et al., 1992), yellowmouth grouper $K$ = 0.08/year (Bullock and Murphy, 1994), and warsaw grouper $K$ = 0.054/year (Manooch and Mason, 1987).

## Sexual maturation and transition

Our histological analysis of black grouper gonads is consistent with the diagnostic criteria of Sadovy and Shapiro (1987) for a monandric protogynous hermaphrodite. Furthermore, the absence of small males and a sex ratio highly skewed towards females are consistent with our diagnosis of protogynous hermaphroditism. Although the length and age distributions of males and females overlapped, males occupied the largest and oldest length and age classes and were unrepresented in smaller and younger length and age classes (Figs. 1 and 6). This is an important difference between black grouper and Nassau grouper (*Epinephelus striatus*), which has recently been diagnosed as a gonochorist with potential for sex change (Sadovy and Colin, 1995). In

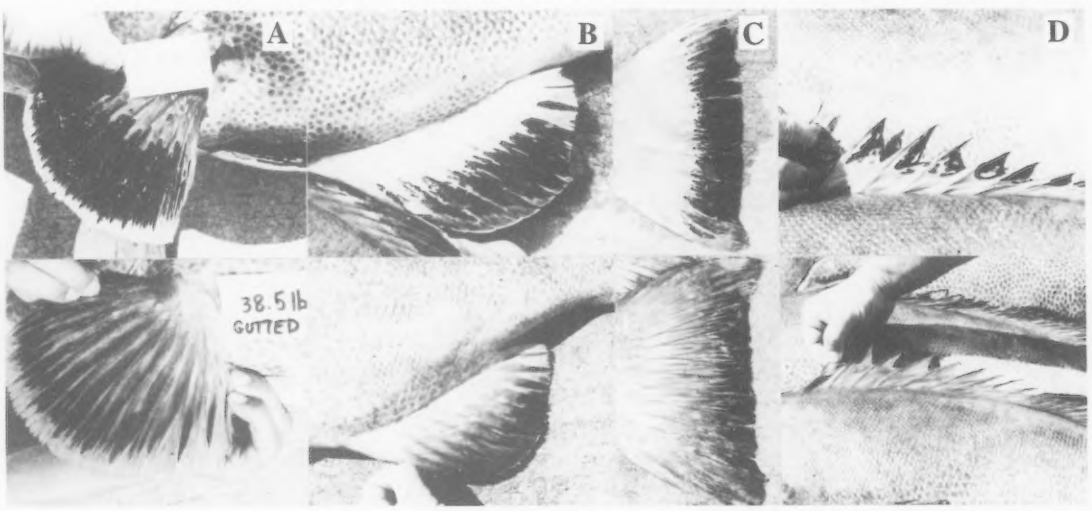


**Figure 10**

Differences in fin pigmentation in male (above) and female (below) black grouper, *Mycteroperca bonaci*. (**A**) Medial surface of pectoral fin, (**B**) anal fin, and (**C**) caudal fin jet black in males. (**D**) Dorsal interspinous membrane yellow or dusky in females, usually black in males.

Nassau grouper, both males and females can develop directly from juveniles and there is little difference in the lengths of males and females. Furthermore, there is little difference in the length at which sexual maturation occurs in both sexes, in contrast to black grouper. The scarcity of transitional black grouper and the absence of immature males in our samples suggest that transition occurs quickly and that testes become active soon after transition. The presence of large females in the population suggests that some females may not transform into males, but even the largest females we observed were considerably smaller and younger than the largest and oldest males (Figs. 1 and 6).

The current 20-inch (508-mm) minimum size limit is well below our estimate of the size at which 50% of females reach sexual maturity (826 mm and 5.2 years) and is not adequate to allow females to reproduce before recruiting to the fishery. Similar estimates of length at maturity were made by García-Cagide and García (1996) who reported that the smallest sexually mature female black grouper they examined from Cuban waters was 570 mm and that most mature females were 850–1100 mm. Huntsman et al. (1994) used an estimate of 5.04 years as the age at sexual maturity in yield per recruit and spawning stock per recruit models for black grouper. They did not present data to support this estimate, but it is within two standard errors of ours.

Female black grouper reached sexual maturity at a relatively large size compared to other grouper species. Female gag reach 50% sexual maturity at 600–650 mm and 3–4 years (Hood and Schlieder, 1992). Female yellowmouth grouper mature between 400 and 450 mm and 2–4 years (Bullock and Murphy, 1994). Yellowedge grouper, *E. flavolimbatus*, reach 50% maturity at 568 mm (Bullock et al., 1996). Larger grouper such as jewfish reach sexual maturity as females at even larger lengths of 1200–1350 mm and greater ages of 6–7 years than black grouper do (Bullock et al., 1992).

The length and age at which 50% of our sample consisted of females was 1215 mm and 15.5 years. García-Cagide and García (1996) reported that the smallest male black grouper from Cuban waters was 980 mm long and that most males were 1000 to 1100 mm long, similar to our findings even though their sample appeared to contain fewer large grouper than ours did. Our estimates of the length and age at transition for black grouper are both larger and older than estimates for other grouper species. Eastern Gulf of Mexico gag populations are 50% male at about 1050 mm and age 11 (Hood and Schlieder, 1992). Gag in the South Atlantic Bight undergo transition from female to male at 750–950 mm and age 5–11 (Collins et al., 1987). Transitional yellowmouth grouper examined by Bullock and Murphy (1994) were 505–643 mm and 5–14 years old. Yellowedge grouper popula-

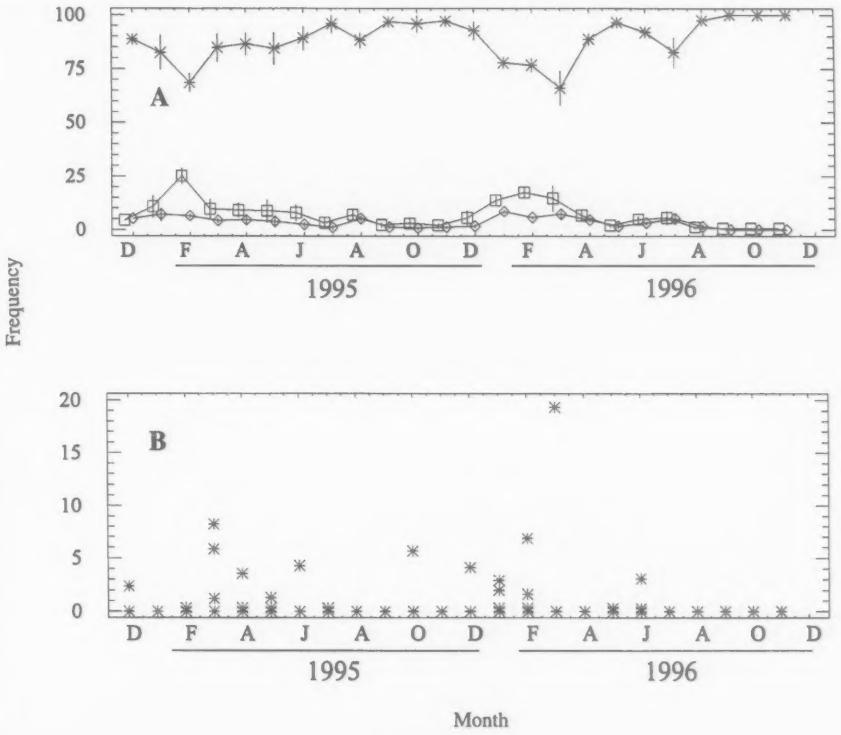


**Figure 11**

(**A**) Monthly mean percent frequency of occurrence and standard errors of oocyte stages in black grouper, *Mycteroperca bonaci*, ovaries ($n$=774) for 1995–96. * = primary growth stage oocytes, = cortical alveolar oocytes, and = vitellogenic oocytes. (**B**) The percent frequency of occurrence of oocytes in the final stages of maturation as a function of month in black grouper ovaries.

tions are 50% females at 817 mm (Bullock et al., 1996). Other large grouper such as warsaw grouper and jewfish have not been confirmed to be protogynous hermaphrodites.

The sex ratio of our sample (1:15.4, male:female) may not resemble that of the population because many black grouper that we examined, especially large fish, were eviscerated and could not be sexed. Most of these large fish were probably males, so we may have underestimated their numbers. We are not able to assess the extent of this bias. Furthermore, sex ratios appear to vary widely depending on the depth and location sampled. It is possible that fishing mortality has reduced the numbers of large males in the population, as has been reported for gag from the eastern Gulf of Mexico, where male:female sex ratios as extreme as 1:76.6 have been reported (Coleman et al., 1996). Unfortunately, there are no historical estimates of black grouper sex ratios with which to compare our estimates. García-Cagide and García (1996) reported a male:female sex ratio of 1:30.3 for black grouper from Cuban waters, more skewed towards females than our overall sex ratio but less skewed than the sex ratio of the black grouper we sampled that were landed in the Florida Keys (1:58.7).

Hydrated black grouper oocytes ranged from 0.8 to 1.2 mm in diameter and are similar in size to the hydrated oocytes reported for other groupers (Moe, 1969; Colin et al., 1987; Carter et al., 1994; Sadovy et al., 1994b). We usually did not know the time of day when fish were caught, so we could not estimate the time at which hydration began. Vitellogenic oocytes reached a diameter of about 0.6 mm before under going the final stages of maturation. A distinct group of vitellogenic oocytes with a mean diameter of about 0.6 mm, along with a clutch of larger hydrated oocytes, was present in most of the six grouper for which we measured oocytes. This is consistent with a group-synchronous mode of reproduction (Wallace and Selman, 1981).

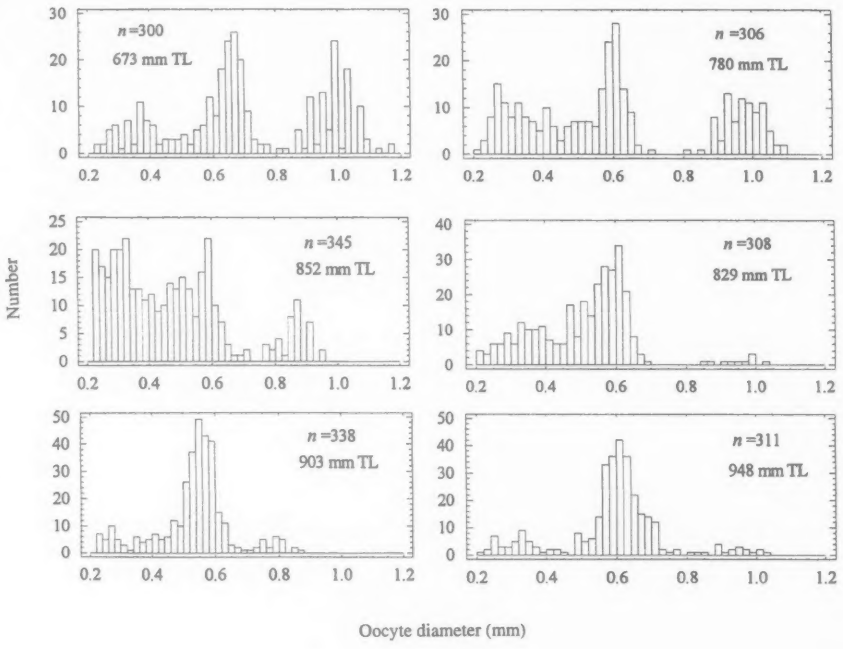


**Figure 12**

Oocyte diameter (mm) frequency distributions for six hydrated female black grouper, *Mycteroperca bonaci*, from South Florida waters. Hydrated oocytes were 0.8–1.2 mm in diameter. Only oocytes with diameters larger than 0.2 mm were measured. *n* = the number of oocytes measured.

## Seasonality of gonad development

Reproduction in black grouper was seasonal, with peak gonad development during December–March, but females with vitellogenic oocytes were present during all months and females with elevated GSIs occurred in most months. In Cuban waters, García-Cagide and García (1996) also found that black grouper spawn during winter and spring. Other eastern Gulf of Mexico grouper species have similar seasonal patterns of gonad development, and in several species, the spawning season appears to be prolonged. In gag, gonad development occurs during December–May, and peak gonadal activity occurs during February and March (Hood and Schlieder, 1992). Yellowmouth grouper gonads are active throughout the year, and peak gonadal activity occurs during April–May (Bullock and Murphy, 1994). Jewfish have developed gonads from June–December, with peak activity during July–September (Bullock et al., 1992). Yellowedge grouper gonads are developed during January–October, with peak activity during May–September (Bullock et al., 1996).

Many grouper species are known to form seasonal spawning aggregations at specific times and locations each year (Sadovy, 1994). Aggregations of black grouper have been reported off Central America during January and February (Carter, 1989; Fine, 1990; Carter et al., 1994), the same time of year that we observed peak gonadal development and the greatest incidence of oocytes in the final stages of maturation. Among other species of *Mycteroperca*, aggregations of gag and scamp have been reported (Gilmore and Jones, 1992), and spawning by an aggregation of tiger grouper, *M. tigris*, was reported by Sadovy et al. (1994a). Spawning aggregations of black grouper have not been documented in Florida waters, but it is clear from the presence of oocytes in the final stages of maturation in our sample that spawning black grouper are landed in South Florida. Whether black grouper form spawning aggregations in Florida waters and the extent of these aggregations is unknown.

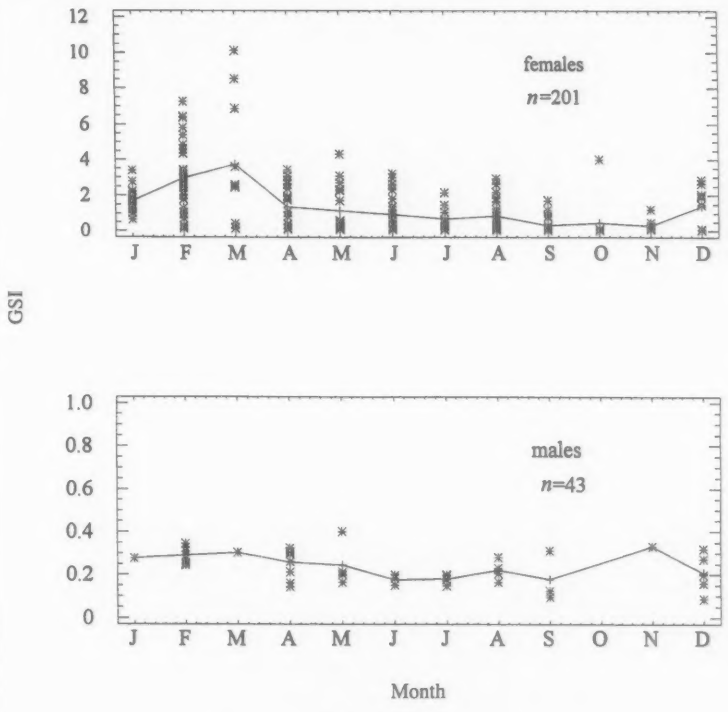


**Figure 13**

Gonadosomatic indices (GSI, *) and medians (+ and lines) for sexually mature female and male black grouper, *Mycteroperca bonaci*, plotted by month.

## Conclusions

The status of black grouper stocks is unclear. Our sample contained many old and large grouper, most of which came from the commercial longline fishery. Large grouper are apparently still abundant in some areas, and their continuing presence in the commercial fishery could reflect the expansion of fishing effort into deeper and more remote areas. In the shallow waters adjacent to the Florida Keys, large and old black grouper were rare, probably a consequence of fishing mortality. The current minimum size does not protect sexually mature females, and the fishery could substantially reduce spawning success in black grouper. In addition, size-selective fishing mortality may selectively remove males from the population. Sex ratios are currently skewed towards females, and it is unknown if populations can compensate by reducing the size of transition as males are selectively removed. Finally, additional research is needed to document the existence of black grouper spawning aggregations in the eastern Gulf of Mexico and in the waters off the Florida Keys and to evaluate the extent to which these aggregations have been affected by fishing mortality.

## Acknowledgments

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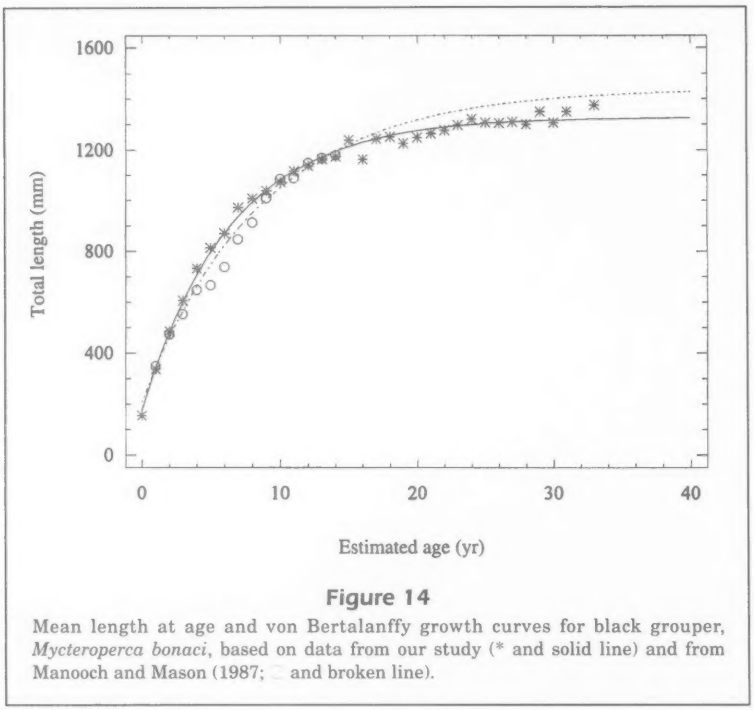


**Figure 14**

Mean length at age and von Bertalanffy growth curves for black grouper, *Mycteroperca bonaci*, based on data from our study (* and solid line) and from Manooch and Mason (1987; ○ and broken line).

Florida Panhandle. J. Leiby, R. McBride, M. Murphy, J. Quinn, and D. Winkelman made helpful comments that improved the manuscript. Lastly, we thank C. Manooch and M. Burton for providing us with the data described in Manooch and Mason (1987). This project was funded by a grant from the National Marine Fisheries Service, U.S. Department of Commerce, Award NA57FF0060, L.H. Bullock and R.E. Crabtree, principal investigators.

## Literature cited

**Bannerot, S. P., W. W. Fox Jr., and J. E. Powers.**
**1987.** Reproductive strategies and the management of snappers and groupers in the Gulf of Mexico and Caribbean. *In* J. J. Polovina and S. Ralston (eds.), Tropical snappers and groupers: biology and fisheries management, p. 561–603. Westview Press, Boulder, CO.

**Bullock, L. H., M. F. Godcharles, and R. E. Crabtree.**
**1996.** Reproduction of yellowedge grouper, *Epinephelus flavolimbatus*, from the eastern Gulf of Mexico. Bull. Mar. Sci. 59:216–224.

**Bullock, L. H., and M. D. Murphy.**
**1994.** Aspects of the life history of the yellowmouth grouper, *Mycteroperca interstitialis*, in the eastern Gulf of Mexico. Bull. Mar. Sci. 55:30–45.

**Bullock, L. H., M. D. Murphy, M. F. Godcharles, and M. E. Mitchell.**
**1992.** Age, growth, and reproduction of jewfish *Epinephelus itajara* in the eastern Gulf of Mexico. Fish. Bull. 90: 243–249.

**Bullock, L. H., and G. B. Smith.**
**1991.** Seabasses (Pisces: Serranidae). Mem. Hourglass Cruises, Vol. 8, part 2, 243 p.

**Carter, J.**
**1989.** Grouper sex in Belize. Nat. Hist. 10:60–69.

**Carter, J., G. J. Marrow, and V. Pryor.**
**1994.** Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proc. Gulf. Caribb. Fish. Inst. 43:65–111.

**Chan, S. T. H., A. Wright, and J. G. Phillips.**
**1967.** The atretic structures in the gonads of the rice-field eel (*Monopterus albus*) during natural sex-reversal. J. Zool. (Lond.) 153:527–539.

**Coleman, F. C., C. C. Koenig, and L. A. Collins.**
**1996.** Reproductive styles of shallow-water groupers (Pisces:Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fish. 47:129–141.

**Colin, P. L., D. Y. Shapiro, and D. Weiler.**
**1987.** Aspects of the reproduction of two groupers, *Epinephelus guttatus* and *E. striatus* in the West Indies. Bull. Mar. Sci. 40:220–230.

**Collins, M. R., C. W. Waltz, W. A. Roumillat, and D. L. Stubbs.**
**1987.** Contribution to the life history and reproductive biology of gag, *Mycteroperca microlepis* (Serranidae), in the South Atlantic Bight. Fish. Bull. 85:648–653.

**Fine, J. C.**
**1990.** Groupers in love. Sea Frontiers. Jan–Feb:42–45.

**García-Cagide, A., and T. García.**
**1996.** Reproducción de *Mycteroperca bonaci* y *Mycteroperca*

*venenosa* (Pisces: Serranidae) en la plataforma Cubana. Rev. Biol. Trop. 44:771–780.

**Gilmore, R. G., and R. S. Jones.**
**1992.** Color variation and associated behavior in the epinepheline groupers *Mycteroperca microlepis* (Goode and Bean) and *M. phenax* (Jordan and Swain). Bull. Mar. Sci. 51:83– 103.

**Hood, P. B., and R. A. Schlieder.**
**1992.** Age, growth, and reproduction of gag, *Mycteroperca microlepis* (Pisces:Serranidae), in the eastern Gulf of Mexico. Bull. Mar. Sci. 51:337–352.

**Hubbs, C. L., and K. F. Lagler.**
**1964.** Fishes of the Great Lakes region. Univ. Michigan Press, Ann Arbor, MI, 213 p.

**Hunter, J. R., B. J. Macewicz, N. C. Lo, and C. A. Kimbrell.**
**1992.** Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90:101–128.

**Huntsman, G. R., J. Potts, and R. W. Mays.**
**1994.** A preliminary assessment of the populations of seven species of grouper (Serranidae, Epinephelinae) in the western Atlantic Ocean from Cape Hatteras, North Carolina to the Dry Tortugas, Florida. Proc. Gulf Caribb. Fish. Inst. 43:193–213.

**Manooch, C. S., III, and D. L. Mason.**
**1987.** Age and growth of the warsaw grouper and black grouper from the southeast region of the United States. Northeast Gulf Sci. 9:65–75.

**Matheson, R. H. III, G. R. Huntsman, and C. S. Manooch III.**
**1986.** Age, growth, mortality, food and reproduction of the scamp, *Mycteroperca phenax*, collected off North Carolina and South Carolina. Bull. Mar. Sci. 38:300–312.

**Moe, M. A., Jr.**
**1969.** Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico. Fla. Dep. Nat. Resour. Mar. Res. Lab. Prof. Pap. ser. 10, 95 p.

**Quintero-Hunter, I., H. Grier, and M. Muscato.**
**1991.** Enhancement of histological detail using metanil yellow as counterstain in periodic acid Schiff's hematoxylin staining of glycol methacrylate tissue sections. Biotechnol. Histochem. 66:169–172.

**Ravaglia, M. A., and M. C. Maggese.**
**1995.** Melano-macrophage centers in the gonads of the swamp eel, *Synbranchus marmoratus* Bloch, (Pisces, Synbranchidae): histological and histochemical characterization. J. Fish Dis. 18:117–125.

**Sadovy, Y.**
**1994.** Grouper stocks of the western central Atlantic: the need for management and management needs. Proc. Gulf Caribb. Fish. Inst. 43:43–64.

**Sadovy, Y., and P. L. Colin.**
**1995.** Sexual development and sexuality in the Nassau grouper. J. Fish Biol. 46:961– 976.

**Sadovy, Y., P. L. Colin, and M. L. Domeier.**
**1994a.** Aggregation and spawning in the tiger grouper, *Mycteroperca tigris* (Pisces: Serranidae). Copeia 1994: 511–516.

**Sadovy, Y., A. Rosario, and A. Roman.**
**1994b.** Reproduction in an aggregating grouper, the red hind, *Epinephelus guttatus*. Environ. Biol. Fish. 41:269–286.

**Sadovy, Y., and D. Y. Shapiro.**
**1987.** Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987:136–156.

**Secor, D. H., J. M. Dean, and E. H. Laban.**
**1992.** Otolith removal and preparation for microstructural examination. *In* D. K. Stevenson and S. E. Campana (eds.), Otolith microstructure examination and analysis, p. 19–57. Can. Spec. Publ. Fish. Aquat. Sci. 117.

**Smith, C. L.**
**1971.** A revision of the American groupers: *Epinephelus* and allied genera. Bull. Am. Mus. Nat. Hist. 146(2):67–242.

**von Bertalanffy, L.**
**1957.** Quantitative laws in metabolism and growth. Q. Rev. Biol. 2:217–231.

**Wallace, R. A., and K. Selman.**
**1981.** Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool. 21:325–343.

# Feeding habits of bonefish, *Albula vulpes*, from the waters of the Florida Keys

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**Abstract**–We examined stomach contents of 385 bonefish that ranged in length from 228 to 702 mm FL. Relatively few prey species made up most of the diet by weight—xanthid crabs (29.9%), gulf toadfish, *Opsanus beta* (17.2%), portunid crabs (10.9%), alpheid shrimp (9.2%), and penaeid shrimp (7.7%) together made up 74.9%. A variety of gastropods (17 families and 24 species) and bivalves (9 families and 16 species) were eaten, but gastropods made up only 2.7% of the diet by weight and bivalves made up only 2.5%. Polychaetes, represented by at least seven families, were important in the diet numerically (37.1%) but made up little of the diet by weight (1.4%). Cluster analysis and ordination of stomach contents permitted bonefish to be grouped according to length. Large bonefish (>439 mm FL) ate more xanthid crabs, alpheid shrimp, *Callinectes* spp., and *O. beta* than did small bonefish; penaeid shrimp were more important in the diet of small bonefish (<440 mm FL). The stomach contents of bonefish caught in Florida Bay were significantly different from those of bonefish caught on the ocean (Florida Straits) side of the Florida Keys, but the differences were slight and the same prey taxa dominated the diet in both areas. Xanthid crabs, alpheid shrimp, *O. beta*, penaeid shrimp, and *Callinectes* spp. together made up over 50% of the dissimilarity in diet of bonefish between the two areas. Some seasonal effects on diet were found, but variable sample sizes among seasons in the respective sampling areas made it difficult to detect seasonal trends. Bonefish fed selectively on some prey groups, but other common prey groups were not selected and were less common in stomachs than in the prey environment. The suite of epibenthic crustaceans and fishes found in bonefish stomachs was significantly different from that available as prey in the environment. Our results suggest that teleosts, mainly *O. beta*, are more important in the diet of bonefish than reported in previous studies.

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Fish. Bull. 96:754–766 (1998).

Bonefish, *Albula vulpes*, are the basis of an economically important recreational fishery in the Florida Keys and many parts of the Caribbean. In the Florida Keys, fishing for bonefish is a year-round activity and provides an important source of income to professional fishing guides. Most bonefish are caught in relatively shallow (<2 m) water over either seagrass or sandy bottom, and it is common for bonefish to forage in water less than 0.3 m deep, where their tails and dorsal fins can often be seen extending from the water as they feed on benthic and epibenthic prey. Bonefish are known for their wariness when approached in shallow water and for their strong fighting abilities when hooked. In Florida, the commercial sale of bonefish is prohibited, and regulations on the recreational fishery include a bag limit of one fish per angler per day and a minimum total length of 457 mm (390 mm fork length). Bonefish are not considered a food fish in Florida, and therefore most bonefish caught are released.

Crabtree et al. (1996, 1997) recently described the age, growth, and reproduction of bonefish from South Florida waters and found that bonefish can attain ages of 19 years. In the Florida Keys, 50% of male bonefish reach sexual maturity at 418 mm and an age of 3.6 years, and 50% of female bonefish reach sexual maturity at 488 mm FL and an age of 4.2 years. Bonefish gonadal activity in the Florida Keys is seasonal and spawning occurs during November–May.

Feeding habits of bonefish have been studied by Warmke and Erdman (1963) in Puerto Rico, by Bruger (1974) in the Florida Keys, and by Colton and Alevizon (1983) in the Bahamas; however, none of these studies have adequately described the diet of bonefish. Warmke and Erdman (1963) identified only mollusks, Bruger (1974) presented frequency of occurrence data for crustaceans but did not quantify noncrustacean prey, and Colton and Alevizon (1983) sorted prey into 10 broad taxonomic categories but did not quantify the abundance of each prey species. Consequently, the relative importance of each prey species in the diet of bonefish is unknown. This information is needed to evaluate the effects of habitat changes on Keys bonefish populations and is particularly important considering the recent seagrass die-offs that have been documented in Florida Bay (Robblee et al., 1991; Carlson et al., 1994; Durako, 1994; Butler et al., 1995; Matheson et al.[1]). If changes in the benthic epifauna and infauna

have resulted from the seagrass die-off, these changes could potentially affect both feeding and occurrence of bonefish in Florida Bay. In this article, we describe the feeding habits of bonefish from waters off the Florida Keys. We consider both length-related and seasonal changes in bonefish diet. In addition, we compare the diets of bonefish collected from two important Keys areas: Florida Bay (including parts of Everglades National Park and adjacent waters) and ocean-side (Florida Straits) fishing areas off the Florida Keys.

## Methods

### Collections

We examined stomach contents of 385 bonefish collected from South Florida waters from December 1991 to April 1995. Most of these bonefish were caught with hook-and-line gear in waters off the Florida Keys, Florida Bay, and Biscayne Bay during daylight hours either by biologists or by a single professional bonefish guide and his anglers. Supplemental collections of small bonefish (<425 mm FL, $n$=22) were made with seines and gill nets of various sizes in waters off the Florida Keys. Bonefish were placed on ice immediately after capture. Fork length (FL) was later measured to the nearest millimeter (mm), stomachs were removed, and the contents preserved in 10% buffered formalin. Contents of individual stomachs were sorted and identified to the lowest possible taxon. Fragments of prey organisms were counted as one, unless countable parts such as eye lenses were found. Weights of prey organisms were measured by blotting prey items on filter paper and weighing them on an analytical balance. The number of individuals of each food type as a percentage of the total number of identifiable prey items (percent numerical abundance, $N$), the percentage of stomachs containing prey in which a particular prey taxa occurred (frequency of occurrence, $F$), and wet weight as a percentage of the total weight of all prey items (percent weight, $W$) were determined. For the larger and more abundant prey taxa (alpheid shrimp, penaeid shrimp, portunid crabs, xanthid crabs, and *Opsanus beta*), we measured prey size to examine the relation between predator and prey size. We measured total length (TL, tip of the rostrum to tip of the uropod) of shrimp, carapace width of crabs, and standard length (SL) of *O. beta*.

[1] Matheson, R. E., D. A. Camp, S. M. Sogard, and K. A. Bjorgo. 1998. Changes in seagrass-associated fish and crustacean communities on Florida Bay mud banks: the effects of recent ecosystem changes? Manuscript in review.

We compared the abundance of prey found in bonefish stomachs with the abundance of benthic and epibenthic crustaceans and fishes from typical Florida Keys bonefish habitat. Information on the abundance of potential prey in Florida Bay is based on meter-square throw-trap collections by Matheson et al.[1] during 1994–96. We used data for Buchanan Bank ($n$=30 collections) in the Atlantic subenvironment as described by Zieman et al. (1989) and followed by Matheson et al.[1] The Atlantic subenvironment, and specifically the Buchanan Bank area sampled by Matheson et al.,[1] is an area where many of our Florida Bay bonefish were caught. Data on prey abundance from ocean-side (Florida Straits) areas of the Florida Keys are from 54 samples that we collected following the methods of Sogard et al. (1987) and Matheson et al.[1] with meter-square throw traps. Ocean-side samples were taken during September 1996 ($n$=14) and January 1997 ($n$=40) at various locations from the middle Keys north to Elliot Key. We sampled areas where we had previously caught bonefish and that appeared to be representative of typical ocean-side bonefish flats. Throw-trap samples were collected over a different time period (1994–97) than that for our bonefish specimens (1991–95), and we assumed for our comparisons that prey availability did not change over this time. If prey abundance changed during 1991–97, this change could have biased our comparisons.

### Data analysis

Nonparametric multivariate techniques were used to analyze feeding data. Similarity matrices were constructed with pairwise Bray-Curtis similarity coefficients (Bray and Curtis, 1957). Square-root-transformed, percent-standardized prey-weight data were used to generate similarities. Prey weight was used for all comparisons except feeding selectivity comparisons, because this measure more closely reflects the energetic importance of a prey species in the diet than does either frequency of occurrence or percent numerical abundance. Percent numerical abundance was used in feeding selectivity comparisons because we were interested in the relative abundance of prey in stomachs and in the environment. Hierarchical agglomerative cluster analysis that incorporated a group-average linking method was used to search for groups among bonefish stomach contents. A nonparametric ordination technique, nonmetric multidimensional scaling (MDS), was used to ordinate sites on the basis of the similarity matrix. The contribution of the various prey categories to the percentage similarity within groups and the differences among groups were estimated with a simi-

larities percentage (SIMPER) procedure (Clarke, 1993; Clarke and Warwick, 1994). All multivariate analyses were performed with Plymouth Routines in Marine Environmental Research (PRIMER) programs (copyright M. R. Carr and K. R. Clarke, Marine Biological Laboratory, Plymouth, UK; Clarke and Warwick, 1994).

Separate analyses were performed to compare stomach contents between various length groups of bonefish, between seasons, and between areas (Table 1). To detect length-related differences in feeding, we pooled bonefish into 20-mm length intervals and used cluster and MDS analyses to compare stomach contents. Stomach contents of all 20-mm length groups within the 480- to 699-mm length range had a level of similarity >55%, so we restricted all other comparisons to this length group in order to minimize length-related dietary shifts that could have confounded comparisons of areas and seasons. We compared stomach contents of bonefish from two areas, Florida Bay and the ocean (Florida Straits) side of the Keys, using the analysis of similarity (ANOSIM) permutation test (Clarke, 1993; Clarke and Warwick, 1994). We did not include the lower Keys or Biscayne Bay in our area comparisons because we examined relatively few bonefish stomachs from these two areas. Bonefish move seasonally between Florida Bay and ocean-side areas; thus for any given month sample sizes were rarely the same for the two areas. To reduce confounding from seasonal effects that could result from unequal seasonal representation of the two areas, we eliminated some stomachs from our analysis to achieve equal monthly sample sizes for the two areas for each month. We pooled samples from all years, totaled the number of samples by month for each area, and then randomly eliminated stomachs for each month from the area with the greatest sample size so that, for any given month, both areas had equal sample sizes. The resulting sample of 50 stomachs from each area contained equal sample sizes from both areas for each month, but the total sample size varied from month to month (Table 1). To detect seasonal dietary shifts, collections were pooled into four seasonal groupings: January–March, April–June, July–September, and October–December. We used ANOSIM to make seasonal comparisons of the diets of bonefish separately for the two principal sampling areas. Six pairwise comparisons were made among seasons for each area. No adjustment of significance levels exists for ANOSIM to account for the increased possibility of type-1 error associated with multiple comparisons (Clarke and Warwick, 1994).

To determine feeding selectivity, we used ANOSIM to compare the species of crustaceans and fishes found in the stomachs of bonefish 480–699 mm long to those found in samples collected in the potential-prey environment. We included only bonefish collected during the same seasons as those when the throw-trap collections were made. For Florida Bay comparisons, bonefish collected during March, May, June, and September were included; for ocean-side comparisons, bonefish collected during January, February, August, September, and October were included (Table 1). Significant differences in the suite of crustaceans and fishes found in bonefish stomachs and the potential prey available in the environment would imply selective feeding. We used SIMPER to indicate the percentage of dissimilarity contributed by each prey species and thus show which prey were selected or not selected. Taxa that were not selected

**Table 1**

Sample sizes, and collection months and years for data included in ANOSIM comparisons. Numbers in parentheses are the number of samples collected during a particular month. Bonefish, *Albula vulpes*, fork lengths for all comparisons ranged from 480 to 699 mm.

| Comparison | *n* | Months | Years |
|---|---|---|---|
| Area | | | |
| Florida Bay | 50 | Jan (5), Feb (3), Mar (2), Apr (5), May (7), Jun (1), Jul (4), Aug (4), Sep (3), Oct (6), Nov (7), Dec (3) | 1991–1995 |
| Ocean side | 50 | same monthly sample sizes as Florida Bay | 1991–1995 |
| Season | | | |
| Ocean side | | | |
| Jan–Mar | 39 | | 1991–1995 |
| Apr–Jun | 43 | | |
| Jul–Sep | 6 | | |
| Oct–Dec | 33 | | |
| Florida Bay | | | 1991–1995 |
| Jan–Mar | 8 | | |
| Apr–Jun | 25 | | |
| Jul–Sep | 70 | | |
| Oct–Dec | 18 | | |
| Stomach throw trap | | | |
| Ocean side | | | |
| Stomachs | 39 | Jan. (7), Feb (13), Aug (4), Sept (3), Oct (12) | 1991–1995 |
| Throw traps | 54 | Jan (40), Sep (14) | 1996–1997 |
| Florida Bay | | | |
| Stomachs | 45 | Mar (2), May (9), Jun (10), Sep (24) | 1991–1995 |
| Throw traps | 30 | Mar (6), May (6), Jun (6), Sep (12) | 1994–1996 |

could have been avoided, not preferred, or only incidentally ingested by bonefish. Alternatively, taxa that were not selected could have been preferred, but were able to evade capture.

## Results

Stomachs of 385 bonefish that ranged in length from 228 to 702 mm contained prey (Fig. 1) consisting mostly of small benthic and epibenthic organisms (Table 2). Stomachs of 67 of the bonefish we examined were empty. Decapods and teleosts dominated the diet by weight, but gastropods and bivalves were among the most speciose prey categories. Relatively few prey species made up most of the diet by weight; xanthid crabs ($W$=29.9%), the gulf toadfish, *Opsanus beta* ($W$=17.2%), portunid crabs ($W$=10.9%), alpheid shrimp ($W$=9.2%), and penaeid shrimp ($W$=7.7%) together made up 74.9% of the diet. At least 17 families and 24 species of gastropods and 9 families and 16 species of bivalves were recognized, but gastropods made up only 2.7% of the diet by weight and bivalves made up only 2.5%. Polychaetes, represented by at least seven families, were important numerically ($N$=37.1%) but made up only 1.4% of the diet by weight.

Both the cluster analysis and the MDS ordination grouped bonefish stomach contents according to fish length (Fig. 2). Cluster analysis organized the 23 length groups into two principal clusters that were linked at a level of similarity greater than 20% and one outlying group of bonefish 280–299 mm long (group 2) that was linked to the other clusters at a level of similarity less than 10%. One principal cluster contained bonefish 260 to 439 mm long, and a second principal cluster contained mostly larger fish 400 to 702 mm long. Bonefish in the 400–419 mm length interval (group 8) were classified with larger fish, and bonefish in the 420–439 mm length interval (group 9) were classified with smaller bonefish. In Table 3, stomach contents are summarized separately for small bonefish (<440 mm) and large bonefish (>439 mm) on the basis of cluster analysis, but we reassigned bonefish stomachs in the 400–419 mm length interval (group 8) into the <440 mm group to avoid any overlap in the table between lengths of small (<440 mm) and large (>439 mm) bonefish. In the SIMPER analysis (Table 4), bonefish stomachs were grouped according to the cluster analysis shown in Figure 2, and no groups were reassigned. Levels of similarity among stomach contents of the 20-mm length groups within the 260–439 mm cluster were less overall than the levels of similarity of stomach contents of the 20-mm length groups of bonefish in the 400–699 mm cluster. Stomach contents of bonefish length groups ranging from 480 to 699 mm had a high level of similarity (>55%) and were tightly grouped in the MDS; we chose this length group as the basis for all other statistical comparisons.

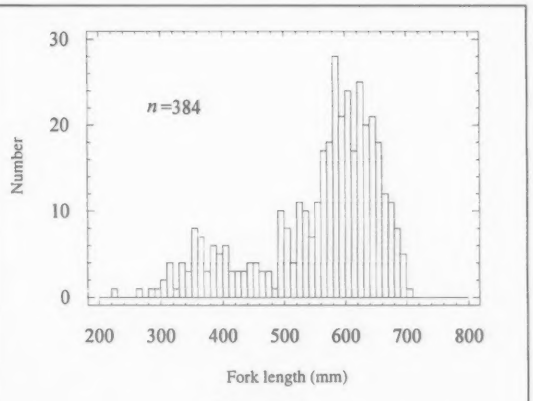


**Figure 1**

Length-frequency distribution of 384 bonefish, *Albula vulpes*, whose stomachs contained recognizable prey. The tail of one of the 385 bonefish whose stomach contents were examined was eaten by a shark during capture, so that fish was not measured.

SIMPER analysis suggested that much of the dissimilarity between the two principal length clusters was due to xanthid crabs, penaeid shrimp, alpheid shrimp, and *O. beta* (Table 4). The large values of the ratios ($\delta_i$ /SD($\delta_i$)) between the mean contribution ($\delta_i$) to the overall level of dissimilarity and the standard deviation (SD) of the $\delta_i$ values across all stomachs suggest that these taxa consistently contributed to the level of dissimilarity, and so they are probably reliable discriminating prey taxa characteristic of one or the other length clusters. Bonefish longer than 439 mm consumed more decapods (alpheid shrimp, xanthid crabs, and *Callinectes* spp.) and teleosts than smaller bonefish (Tables 3 and 4). The most striking difference was in the consumption of teleosts, principally *O. beta*, which was not eaten by bonefish shorter than 440 mm but made up 17.8% of the diet of bonefish longer than 439 mm. Penaeid shrimp made up a larger proportion of the diet of small bonefish ($W$=40.5) than large bonefish ($W$=6.7), but they were eaten by bonefish of all sizes. Portunid crabs were eaten in nearly equal amounts by both length groups of bonefish, but this finding is misleading because all the *Portunus* spp. eaten by small bonefish were eaten by a single individual collected in Florida Bay; thus the importance of portunid crabs in the diet of small bonefish is probably less than what is suggested in Table 3. No crabs of the genus *Callinectes* were eaten by small bonefish.

**Table 2**

Food items found in stomachs of bonefish, *Albula vulpes*, caught in the waters of the Florida Keys ($n$=385). $W$ = percent weight, $F$ = percent frequency of occurrence, $N$ = percent numerical abundance.

| Taxon and prey item | $W$ | $F$ | $N$ |
|---|---|---|---|
| Plant material | 0.6 | 34.5 | — |
| Algae | <0.1 | 0.5 | — |
| *Halimeda* | <0.1 | 8.1 | — |
| Unidentifiable seagrass | 0.1 | 8.1 | — |
| *Halodule* | <0.1 | 7.0 | — |
| *Syringodium* | <0.1 | 1.6 | — |
| *Thalassia* | 0.3 | 19.2 | — |
| Annelida | | | |
| Total Polychaeta | 1.4 | 39.5 | 37.1 |
| Unidentified Polychaeta | 0.2 | 6.8 | 1.7 |
| Amphinomidae | 0.3 | 1.3 | <0.1 |
| Lumbrinereidae | | | |
| *Lumbrinereis* spp. | <0.1 | 0.8 | 0.7 |
| Opheliidae | 0.7 | 30.1 | 33.0 |
| Spionidae | <0.1 | 0.8 | 0.4 |
| Orbiniidae | <0.1 | 1.3 | 0.9 |
| Capitellidae | | | |
| Unidentified Capitellidae | <0.1 | 1.6 | 0.1 |
| *Dasybranchus* spp. | <0.1 | 0.5 | <0.1 |
| Sabellidae | <0.1 | 1.0 | 0.1 |
| Mollusca | | | |
| Unidentified Mollusca | 1.6 | 15.6 | 0.8 |
| Total Gastropoda | 2.7 | 31.2 | 5.9 |
| Unidentified Gastropoda | 0.3 | 8.6 | 0.6 |
| Acmaeidae | | | |
| Unidentified Acmaeidae | <0.1 | 0.3 | <0.1 |
| *Patelloida pustulata* | <0.1 | 0.3 | <0.1 |
| Trochidae | | | |
| *Tegula fasciata* | <0.1 | 0.3 | <0.1 |
| Turbinidae | | | |
| *Turbo castanea* | <0.1 | 0.3 | <0.1 |
| *Eulithidium affine* | <0.1 | 1.3 | 0.5 |
| Turritellidae | | | |
| *Torcula acropora* | <0.1 | 0.3 | <0.1 |
| Modulidae | | | |
| *Modulus modulus* | <0.1 | 1.8 | 0.1 |
| Cerithiidae | | | |
| Unidentified Cerithiidae | <0.1 | 1.0 | <0.1 |
| *Cerithium eburneum* | <0.1 | 0.8 | <0.1 |
| *Cerithium muscarum* | <0.1 | 0.5 | <0.1 |
| Triviidae | | | |
| *Trivia quadripunctata* | <0.1 | 0.5 | <0.1 |
| Naticidae | | | |
| *Natica canrena* | <0.1 | 0.8 | <0.1 |
| Columbellidae | | | |
| Unidentified Columbellidae | <0.1 | 0.3 | <0.1 |
| *Anachis avara* | <0.1 | 1.0 | <0.1 |
| *Columbella rusticoides* | <0.1 | 0.3 | <0.1 |
| *Zafrona taylorae* | <0.1 | 0.5 | <0.1 |
| Nassariidae | | | |
| *Nassarius* sp. | <0.1 | 0.3 | <0.1 |
| Fasciolariidae | | | |
| *Leucozonia nassa* | <0.1 | 0.3 | <0.1 |
| *Fasciolaria tulipa* | 1.5 | 6.8 | 0.6 |
| *Fasciolaria* spp. | <0.1 | 0.5 | <0.1 |
| Olividae | | | |
| Unidentified Olividae | <0.1 | 0.8 | <0.1 |
| *Jaspidella jaspidea* | 0.2 | 8.3 | 0.7 |
| Marginellidae | | | |
| Unidentified Marginellidae | <0.1 | 1.3 | <0.1 |
| *Prunum apicinum* | 0.3 | 8.1 | 1.7 |
| *Prunum* sp. | <0.1 | 1.3 | <0.1 |
| *Volvarina avena* | <0.1 | 1.6 | 0.1 |
| Cystiscidae | | | |
| *Persicula catenata* | <0.1 | 1.6 | <0.1 |
| *Persicula pulcherrima* | <0.1 | 0.3 | <0.1 |
| Conidae | | | |
| *Conus stearnsi* | <0.1 | 0.3 | <0.1 |
| Bullidae | | | |
| *Bulla striata* | <0.1 | 2.3 | 0.7 |
| Nudibranchia | <0.1 | 0.3 | <0.1 |
| Total Bivalvia | 2.5 | 24.9 | 2.9 |
| Unidentified Bivalvia | 0.5 | 5.2 | 0.4 |
| Mytilidae | | | |
| *Brachidontes modiolus* | <0.1 | 0.5 | 0.3 |
| Pteriidae | | | |
| *Pinctada imbricata* | <0.1 | 0.5 | <0.1 |
| Limidae | | | |
| *Limaria pellucida* | 0.1 | 3.9 | 0.6 |
| Pectinidae | | | |
| Unidentified Pectinidae | <0.1 | 0.8 | <0.1 |
| *Argopecten irradians* | 0.5 | 2.1 | 0.1 |
| *Argopecten* spp. | <0.1 | 0.8 | <0.1 |
| Lucinidae | | | |
| Unidentified Lucinidae | 0.2 | 1.6 | 0.1 |
| *Codakia orbicularis* | <0.1 | 0.3 | <0.1 |
| *Codakia orbiculata* | <0.1 | 0.3 | <0.1 |
| Carditidae | | | |
| *Carditamera floridana* | <0.1 | 1.3 | <0.1 |
| Cardiidae | | | |
| Unidentified Cardiidae | <0.1 | 0.5 | <0.1 |
| *Americardia media* | <0.1 | 0.3 | <0.1 |
| *Laevicardium mortoni* | <0.1 | 0.3 | <0.1 |
| *Trachycardium muricatum* | <0.1 | 0.3 | <0.1 |
| Tellinidae | | | |
| Unidentified Tellinidae | <0.1 | 1.0 | <0.1 |
| *Strigilla carnaria* | <0.1 | 0.3 | <0.1 |
| *Tellina fausta* | <0.1 | 0.3 | 0.1 |
| *Tellina similis* | <0.1 | 3.9 | 0.3 |
| *Tellina tampaensis* | <0.1 | 0.5 | 0.1 |
| *Tellina* spp. | <0.1 | 1.3 | <0.1 |
| Veneridae | | | |
| *Chione cancellata* | 0.6 | 6.5 | 0.4 |
| *Transennella conradina* | <0.1 | 0.3 | <0.1 |
| Crustacea | | | |
| Unidentified Crustacea | <0.1 | 1.6 | <0.1 |
| Copepoda | <0.1 | 0.3 | <0.1 |
| Total Stomatopoda | 2.0 | 10.9 | 1.0 |
| Unidentified Stomatopoda | 1.0 | 5.7 | 0.6 |
| *Pseudosquilla ciliata* | 1.0 | 5.2 | 0.3 |

continued

There was a significant positive correlation between prey size and bonefish length for xanthid crabs ($n$=286, $r$=0.262, $P$<0.001) and portunid crabs ($n$=58, $r$=0.465, $P$<0.001), but not for alpheid shrimp ($P$=0.630), penaeid shrimp ($P$=0.063), or *O. beta* ($P$=0.782). The largest prey consumed were *O. beta* (largest 113 mm SL), the portunid crab *Callinectes sapidus* (largest 106 mm carapace width), and penaeid shrimp (largest 100 mm TL; Fig. 3). Most of these relatively large animals were consumed only as juveniles by bonefish.

The stomach contents of bonefish caught in Florida Bay were significantly different from those of bonefish caught on the ocean side of the Keys (ANOSIM, $R$=0.034; $P$=0.013). The statistic $R$ can range from −1 to 1 with a value of 1 indicating that all replicates within a sample are more similar to each other than to any replicates from the other samples and with a value of 0 indicating that the similarities between and within samples are on average equal (Clarke and Warwick, 1994). Although the $R$ value of 0.034 was

**Table 2 (continued)**

| Taxon and prey item | *W* | *F* | *N* |
|---|---|---|---|
| Total Decapoda | 67.8 | 88.6 | 42.1 |
| Unidentified Dendrobranchiata | 0.2 | 8.3 | 0.9 |
| Penaeidae | | | |
| Unidentified Penaeidae | 0.9 | 4.9 | 1.2 |
| *Penaeus* spp. | 3.8 | 17.9 | 2.9 |
| *Penaeus duorarum* | 3.0 | 5.5 | 1.1 |
| Palaemonidae | | | |
| Unidentified Palaemonidae | <0.1 | 8.6 | 1.1 |
| *Brachycarpus biunguiculatus* | <0.1 | 0.8 | <0.1 |
| Alpheidae | | | |
| Unidentified Alpheidae | 0.9 | 9.4 | 1.8 |
| *Alpheus floridanus* | <0.1 | 1.0 | <0.1 |
| *Alpheus normanni* | 7.6 | 35.3 | 13.2 |
| *Alpheus* spp. | 0.6 | 6.8 | 0.9 |
| Hippolytidae | | | |
| Unidentified Hippolytidae | 0.1 | 4.7 | 1.2 |
| *Thor* spp. | 0.3 | 19.7 | 5.6 |
| *Tozeuma* spp. | <0.1 | 0.5 | <0.1 |
| Palinuridae | | | |
| *Panulirus argus* | 0.6 | 0.5 | <0.1 |
| Unidentified Brachyura | 3.8 | 17.7 | 1.0 |
| Majidae | | | |
| Unidentified Majidae | 3.0 | 6.2 | 0.6 |
| *Pitho mirabilis* | 0.2 | 0.3 | <0.1 |
| *Pitho* spp. | 0.7 | 2.3 | 0.2 |
| Portunidae | | | |
| Unidentified Portunidae | 2.9 | 8.1 | 0.6 |
| *Callinectes ornatus* | 1.3 | 1.0 | 0.2 |
| *Callinectes sapidus* | 2.5 | 1.8 | 0.2 |
| *Callinectes* spp. | 3.1 | 7.0 | 0.5 |
| *Portunus* spp. | 1.1 | 1.0 | 0.5 |
| Xanthidae | | | |
| Unidentified Xanthidae | 29.9 | 49.6 | 8.2 |
| *Neopanope* sp. | <0.1 | 0.3 | <0.1 |
| *Panopeus* spp. | 0.1 | 0.8 | 0.1 |
| Grapsidae | | | |
| Unidentified Grapsidae | <0.1 | 1.0 | 0.1 |
| *Sesarma sp.* | <0.1 | 0.3 | <0.1 |
| Palicidae | | | |
| Unidentified Palicidae | <0.1 | 0.3 | <0.1 |
| Mysidae | <0.1 | 0.5 | <0.1 |
| Tanaidacea | <0.1 | 2.9 | 4.0 |
| Isopoda | <0.1 | 0.3 | <0.1 |
| Echinodermata | | | |
| Ophiuroidea | 0.5 | 1.8 | 1.0 |
| Holothuroidea | 0.5 | 2.9 | 0.2 |
| Ascidiacea | <0.1 | 0.3 | <0.1 |
| Chordata | | | |
| Total Teleostei | 20.5 | 44.9 | 4.9 |
| Unidentified teleostei | 0.5 | 10.1 | 0.6 |
| Ophichthidae | | | |
| Unidentified Ophichthidae | 0.2 | 1.3 | 0.1 |
| *Ahlia egmontis* | 0.7 | 2.9 | 0.2 |
| *Myrophis punctatus* | 0.7 | 2.6 | 0.1 |
| Engraulidae | | | |
| Unidentified Engraulidae | <0.1 | 0.3 | <0.1 |
| *Anchoa* sp. | <0.1 | 0.3 | <0.1 |
| Batrachoididae | | | |
| *Opsanus beta* | 17.2 | 29.1 | 3.1 |
| Bythitidae | | | |
| *Ogilbia cayorum* | <0.1 | 0.8 | <0.1 |
| Cyprinodontidae | | | |
| *Floridichthys carpio* | <0.1 | 0.3 | <0.1 |
| *Lucania parva* | <0.1 | 1.0 | <0.1 |
| Syngnathidae | | | |
| Unidentified Syngnathidae | <0.1 | 1.6 | <0.1 |
| *Hippocampus zosterae* | <0.1 | 0.3 | <0.1 |
| *Hippocampus* sp. | <0.1 | 0.3 | <0.1 |
| *Syngnathus floridae* | <0.1 | 0.5 | <0.1 |
| *Syngnathus scovelli* | <0.1 | 0.3 | <0.1 |
| *Syngnathus* spp. | 0.1 | 1.6 | 0.1 |
| Lutjanidae | | | |
| *Lutjanus griseus* | 0.1 | 0.3 | <0.1 |
| Scaridae | 0.2 | 0.8 | <0.11 |
| Gobiidae | | | |
| Unidentified Gobiidae | <0.1 | 1.3 | <0.1 |
| *Gobiosoma robustum* | <0.1 | 0.3 | <0.1 |
| Balistidae | | | |
| *Monacanthus ciliatus* | <0.1 | 0.3 | <0.1 |
| *Monacanthus hispidus* | 0.2 | 0.8 | <0.1 |
| Ostraciidae | | | |
| *Lactophrys* sp. | 0.1 | 0.3 | <0.1 |
| Miscellaneous material | — | 28.1 | — |
| Nonfood material | | | |
| sandy debris | — | 1.8 | — |
| coral rock | — | 0.3 | — |

significantly different from zero, the difference was small and thus the differences between the stomach contents of Florida Bay and ocean-side bonefish were probably slight. Xanthid crabs, alpheid shrimp, *O. beta*, penaeid shrimp, and *Callinectes* spp. together made up over 50% of the dissimilarity between the two areas (Table 5). Although these taxa contributed to the overall level of dissimilarity, the ratios ($\delta_i$/SD($\delta_i$)) between the mean contribution ($\delta_i$) to the overall level of dissimilarity and the standard deviation of the δ values across all stomachs were low for each prey taxa. Thus, these taxa did not consistently contribute to the level of dissimilarity, and are probably not reliable discriminating prey taxa characteristic of either area. In both areas, the same prey taxa dominated the diet (Table 6).

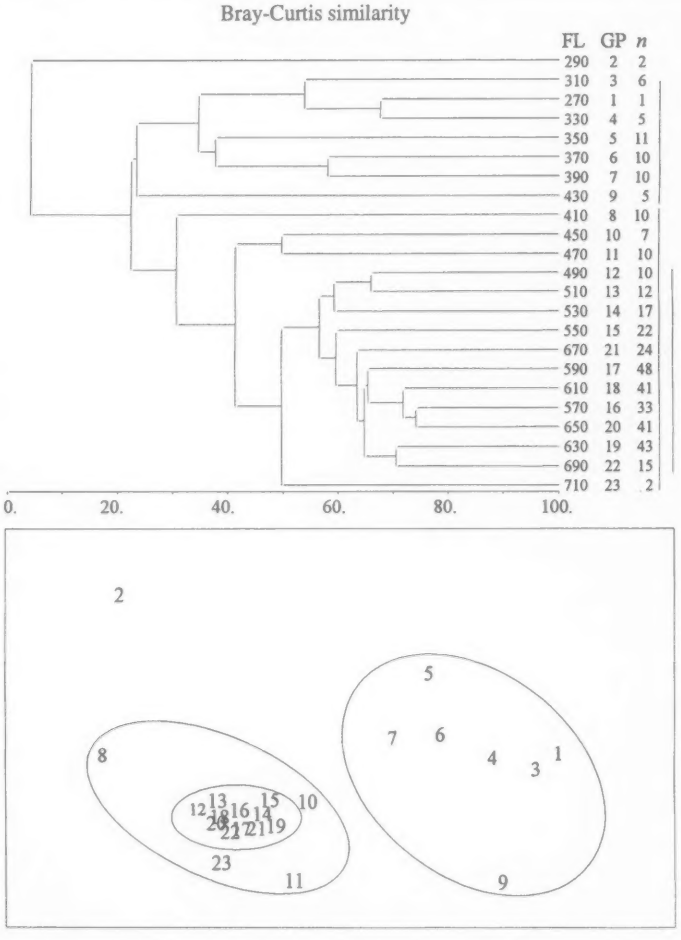


**Figure 2**

Dendrogram and multidimensional scaling (MDS) ordination showing similarities in diet among bonefish, *Albula vulpes*, grouped into 20-mm length intervals. Stress for the MDS plot = 0.13. FL = median fork length of the 20-mm length intervals, GP = group number used on the MDS ordination, and $n$ = the number of bonefish in each 20-mm length interval. The vertical bars to the right of the cluster show the two principal length groupings referred to in the text and the 480–699 mm length grouping used for all statistical comparisons. The circled groups in the MDS ordination correspond to the principal groupings shown on the cluster dendrogram and referred to in the text.

A seasonal effect on feeding was found for ocean-side bonefish (ANOSIM, $R$=0.069; $P$=0.002) but not for bonefish collected from Florida Bay (ANOSIM, $R$=0.066; $P$=0.080). For both tests, the $R$ values were close enough to zero to suggest that any seasonal differences in diet were slight. On the ocean side of the Keys, pairwise tests between bonefish caught during January–March and those caught during all other seasons were significant at $P$<0.05; no other pairwise seasonal comparisons were significant. Xanthid crabs, alpheid shrimp, brachyuran crabs (excluding xanthids, portunids, and majids), *O. beta*, penaeid shrimp, and stomatopods accounted for most of the dissimilarity between stomach contents of bonefish collected during January–March and those of bonefish collected during other seasons (Table 7). The ratios ($\delta_i$/SD($\delta_i$)) between the mean contribution ($\delta_i$) to the overall level of dissimilarity and the standard deviation of the values across all stomachs were low for each prey taxa. No taxa consistently contributed to the level of dissimilarity, and there were no reliable discriminating prey taxa characteristic of any particular season. Variable sample sizes between seasons in both areas reduced the power of our seasonal comparisons; most ocean-side bonefish were caught during January–May, and most Florida Bay bonefish were caught during June–December. Only six stomachs were examined from ocean-side bonefish captured during July–September. Although the stomach contents of these six bone-

**Table 3**

Food items found in stomachs of bonefish, *Albula vulpes*, caught in the waters of the Florida Keys ($n$=384) by bonefish length interval. $W$ = percent weight, $F$ = percent frequency of occurrence, $N$ = percent numerical abundance.

| Taxon and prey item | <440 mm FL ($n$=611) | | | >439 mm FL ($n$=323) | | |
|---|---|---|---|---|---|---|
| | $W$ | $F$ | $N$ | $W$ | $F$ | $N$ |
| Annelida | | | | | | |
| Polychaeta | 3.06 | 19.67 | 9.54 | 1.34 | 43.34 | 40.08 |
| Mollusca | | | | | | |
| Unidentified Mollusca | 2.29 | 19.67 | 1.82 | 1.56 | 14.24 | 0.67 |
| Gastropoda | 4.99 | 21.31 | 13.18 | 2.53 | 32.82 | 5.07 |
| Bivalvia | 4.64 | 22.95 | 3.51 | 2.39 | 25.70 | 2.81 |
| Crustacea | | | | | | |
| Stomatopoda | 2.26 | 3.28 | 2.52 | 2.03 | 12.38 | 0.79 |
| Decapoda | | | | | | |
| Penaeidae | 40.45 | 36.07 | 11.50 | 6.68 | 26.01 | 4.37 |
| Alpheidae | 0.35 | 8.20 | 1.12 | 9.36 | 51.70 | 17.52 |
| Hippolytidae | 1.87 | 11.48 | 7.99 | 0.33 | 26.01 | 6.76 |
| Majidae | 3.28 | 8.20 | 0.70 | 3.92 | 8.98 | 0.89 |
| Portunidae | 15.64 | 8.20 | 3.37 | 10.91 | 19.50 | 1.83 |
| Xanthidae | 7.43 | 13.11 | 1.68 | 31.49 | 56.97 | 8.94 |
| Chordata | | | | | | |
| Teleostei | 3.90 | 19.67 | 3.09 | 21.10 | 49.54 | 5.07 |
| Batrachoididae | | | | | | |
| *Opsanus beta* | 0.00 | 0.00 | 0.00 | 17.83 | 34.67 | 3.43 |

fish were significantly different from those of bonefish collected in January–March (ANOSIM, $R$=0.284, $P$=0.018), we have little confidence in this test because of the small sample size, and these results are not included in Table 7.

Bonefish fed selectively on some prey groups but did not select others. The suite of epibenthic crustaceans and fishes found in bonefish stomachs was significantly different from that collected with throw traps both on the ocean side of the Florida Keys (ANOSIM, $R$=0.261, $P$<0.001) and in Florida Bay (ANOSIM, $R$=0.419, $P$<0.001). Bonefish on the ocean side of the Florida Keys fed selectively on alpheid shrimp, xanthid crabs, *P. duorarum*, and *O. beta*, whereas they did not select the small but abundant crustaceans *Thor* spp. and *Periclimenes americanus* (Table 8). Similarly, Florida Bay bonefish fed selectively on xanthid crabs, alpheid shrimp, *O. beta, P. duorarum,* and *Callinectes* spp. but did not select the abundant but small crustaceans *Thor* spp., *Hippolyte zostericola*, and *P. americanus*, as well as the abundant goby *Gobiosoma robustum* (Table 9).

## Discussion

A variety of factors could have biased our description of the diet of bonefish. Some prey, particularly soft-bodied prey, may have been digested more rapidly than others with bony or chitinous skeletons. Consequently, we may have underestimated the importance of soft-bodied organisms such as polychaetes. Furthermore, bonefish have massive pharyngeal tooth plates capable of crushing shells and other hard structures. If bonefish are able to expel the crushed shells of mollusks and swallow only the soft-bodied organism, then we could have underestimated the importance of mollusks. This might explain why mollusks were relatively unimportant in our samples. Our sample consisted principally of bonefish caught with hook-and-line gear; therefore most of the fish we examined were probably actively foraging or they would not have consumed the bait presented by anglers. We do not believe that the number of fish with empty guts in our sample reflects the number of fish in the area that were not feeding, therefore we did not attempt to evaluate temporal feeding patterns. We cannot eliminate the possibility that some bonefish regurgitated prey during capture trauma; if some prey taxa were more likely to be regurgitated than others, this could have biased our results. Most of the bonefish in our sample came from relatively shallow (<2 m) grass, sand, or hard-bottom flats, but because the fish were caught by anglers, we did not have corresponding data on bottom type for each fish. Colton and Alevizon (1983) found differences in the

**Table 4**

Breakdown into the most important prey groups of the mean dissimilarity between stomach contents (percent weight) of bonefish, *Albula vulpes*, from the two principal clusters shown in Figure 2. Small bonefish ranged from 260 to 439 mm FL and large bonefish ranged from 400 to 699 mm FL. Prey groups are listed in order of decreasing contribution to the overall dissimilarity between the two bonefish length groups. $\delta_i$ is the mean contribution of the *i*th species to the dissimilarity between the two groups, $\delta_i / SD(\delta_i)$ is the ratio between the mean contribution of the *i*th species ($\delta_i$) and the standard deviation of the values for that species [$SD(\delta_i)$], $\delta_i$ % is the contribution to the total dissimilarity scaled as a percentage, and Cum $\delta_i$ % is the cumulative contribution to the total dissimilarity scaled as a percentage. Taxa that are likely to be reliable discriminators of the two length groups are indicated by ** in the $\delta_i /SD(\delta_i)$ column. Taxa proportionally more important in the diet of large bonefish than small bonefish are shown in bold type.

| Species | $\delta_i$ | $\delta_i/SD(\delta_i)$ | $\delta_i$ % | Cum $\delta_i$ % |
|---|---|---|---|---|
| Penaeidae | 8.13 | 1.94** | 10.47 | 10.47 |
| **Xanthidae** | 7.97 | 2.48** | 10.26 | 20.73 |
| **Alpheidae** | 5.29 | 1.84** | 6.81 | 27.54 |
| ***O. beta*** | 5.28 | 1.84** | 6.80 | 34.34 |
| **Portunidae (unidentified)** | 4.62 | 1.26 | 5.95 | 40.29 |
| Brachyura[1] | 3.68 | 1.37 | 4.73 | 45.02 |
| Majidae | 3.25 | 1.05 | 4.19 | 49.21 |
| *Portunus* spp. | 2.80 | 0.45 | 3.60 | 52.81 |
| Stomatopoda | 2.51 | 1.16 | 3.23 | 56.03 |
| ***Callinectes* spp.** | 2.50 | 0.97 | 3.23 | 59.26 |

[1] Excluding xanthids, portunids, and majids.

**Table 5**

Breakdown into the most important prey groups of the mean dissimilarity between stomach contents (percent weight) of bonefish, *Albula vulpes* (480–699 mm FL), caught on the ocean side of the Florida Keys ($n$=50) and in Florida Bay ($n$=50). Prey groups are listed in order of decreasing contribution to the overall dissimilarity between the two study areas. Taxa proportionally more important in the diet of ocean-side bonefish than Florida Bay bonefish are shown in bold type. The low values of $\delta_i /SD(\delta_i)$ suggest that the data were variable and that no taxa were reliable discriminators of either area. Symbols are explained in the legend of Table 4.

| Species | $\delta_i$ | $\delta_i/SD(\delta_i)$ | $\delta_i$ % | Cum $\delta_i$ % |
|---|---|---|---|---|
| **Xanthidae** | 13.47 | 1.15 | 17.17 | 17.17 |
| **Alpheidae** | 8.56 | 1.06 | 10.91 | 28.08 |
| ***O. beta*** | 8.04 | 0.82 | 10.24 | 38.32 |
| Penaeidae | 7.49 | 0.82 | 9.55 | 47.87 |
| *Callinectes* spp. | 4.53 | 0.46 | 5.78 | 53.65 |

stomach contents of Bahamian bonefish collected over different bottom types, and this variation probably also occurs in the Florida Keys. There was also no evidence that bonefish do not feed in deeper waters than those traditionally fished by anglers; prey availability and bonefish feeding may be quite different at greater depths than in the shallow waters we sampled.

Most (77%) of the fish in our sample were longer than 500 mm (Fig. 1); consequently, the diet of large bonefish is better described by our data than that of small bonefish. The inadequacy of our description of the diet of small bonefish is reflected in the low levels of similarity among 20-mm length intervals of bonefish smaller than 480 mm (Fig. 2). Many of the length intervals smaller than 500 mm contained few fish and resulted in greater variation in diet among length intervals and probably caused the lower levels of similarity among 20-mm length groups of small bonefish than among large fish.

The changes in diet as length of bonefish increased in general reflect the expansion of the diet to include larger prey such as *O. beta* and crabs of the genus *Callinectes*. In some cases, for example xanthid and portunid crabs, prey size was positively correlated with predator length. In other cases, for example *O. beta*, prey were not eaten at all by small bonefish, and there was no correlation between prey size and predator length among large bonefish. Small but abundant crustaceans such as *Thor* spp., *H. zostericola*, and *P. americanus* were not important in the diet of the bonefish we examined. These small crustaceans may be eaten by smaller bonefish (<228 mm), but they are apparently outside of the size range of prey typically consumed by the size of bonefish we considered.

Bruger (1974) examined the stomachs of 129 bonefish ranging from 221 to 679 mm FL (reported as 210 to 656 mm SL) collected from the waters off the Keys and reported the frequency of occurrence of crustaceans in the diet. Of these 129 stomachs, 19 were empty. We recalculated his frequency of occurrences on the basis of only the number of stomachs with prey ($n$=110) to compare with our frequency data: the recalculated results were 85% crustaceans, 33% mollusks, and 17% teleosts. These results are in general agreement with our findings of 89% crustaceans, 51% mollusks, and 45% teleosts, except that our samples contained more teleosts than Bruger's. Among crustacean prey, Bruger's results resemble ours; penaeid shrimp, alpheid shrimp, portunid crabs, and xanthid crabs were the most frequently occurring crustacean prey. Although Bruger did not quantify by species the fishes found in stomachs, he did not include *O. beta* in his list of teleosts eaten by

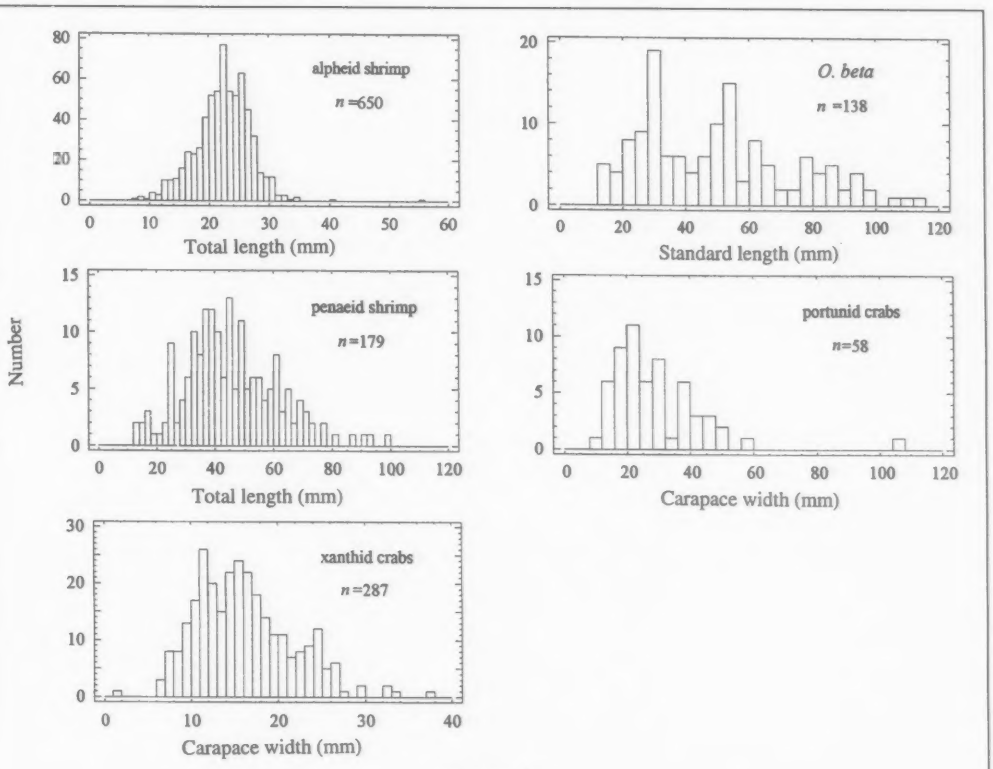


**Figure 3**

Size-frequency distributions of some prey important in the diet of bonefish, *Albula vulpes*.

bonefish. In contrast, *O. beta* was the teleost most frequently eaten by the bonefish we examined ($F$=29.1%). Most of Bruger's bonefish were collected in the lower Keys between Marathon and Key West, but most of our bonefish were captured in the upper Keys from Marathon north to Key Biscayne and including Florida Bay. Although we have no data on prey availability in the lower Keys, habitat differences between the two study areas could account for some of the differences between our results and Bruger's.

Colton and Alevizon (1983) examined the stomach contents of 365 Bahamian bonefish ranging from 268 to 652 mm FL (reported as 256 to 630 mm SL). Bivalves made up 39.2% of the diet of Bahamian bonefish by dry weight, but they made up only 2.5% of the diet of Keys bonefish by weight. Bivalves were the most important prey of Bahamian bonefish both in terms of dry weight (39.2%) and frequency of occurrence (66.3%); portunid ($W$=20.1%; $F$=40.5%) and xanthid crabs ($W$=15.0%; $F$=24.8%) were also important. Teleosts (*O. beta* and *Bathygobius soporator*; pooled $W$=4.9%), alpheid shrimp ($W$=4.6%), *Pseudosquilla ciliata* ($W$=3.2%), polychaetes ($W$=3.2%), gastropods ($W$=2.4%), and *Penaeus duorarum* ($W$=1.6%) occurred in 15–25% of the guts that Colton and Alevizon examined but made up little of the diet in terms of dry weight. The most notable difference between Keys and Bahamian bonefish diets was the greater importance of *O. beta* in the diet of Keys bonefish ($W$=17.2%).

Colton and Alevizon (1983) reported length-related changes in the diet of Bahamian bonefish that were similar to those that we observed in the Florida Keys. They found that bonefish larger than 416 mm FL (400 mm SL) ate more xanthid and majid crabs, alpheid shrimp, and teleosts than smaller bonefish did. Teleosts (gobiids, batrachoidids, ophichthids, and small lutjanids) were found principally in stomachs from bonefish larger than 575 mm FL (555 mm SL). In contrast to our conclusions, Colton and Alevizon (1983) found that small bonefish (<416 mm) ate more portunid crabs (*Callinectes ornatus*) than large bonefish did; we found no *Callinectes* spp. in any bone-

**Table 6**

Food items found in stomachs of bonefish, *Albula vulpes*, caught in Florida Bay ($n$=130) and on the ocean side ($n$=144) of the Florida Keys. Stomachs from all bonefish 480 to 699 mm FL collected during all months are included. $W$ = percent weight; $F$ = percent frequency of occurrence; $N$ = percent numerical abundance.

| Taxon and prey item | Florida Bay | | | Ocean side | | |
|---|---|---|---|---|---|---|
| | *W* | *F* | *N* | *W* | *F* | *N* |
| Annelida | | | | | | |
| Polychaeta | 0.62 | 34.62 | 34.22 | 1.63 | 52.08 | 32.72 |
| Mollusca | | | | | | |
| Gastropoda | 1.86 | 33.85 | 4.94 | 2.83 | 32.64 | 3.52 |
| Bivalvia | 2.68 | 28.46 | 3.00 | 2.02 | 24.13 | 1.85 |
| Crustacea | | | | | | |
| Stomatopoda | 0.35 | 3.85 | 0.24 | 3.85 | 19.44 | 0.73 |
| Decapoda | | | | | | |
| Penaeidae | 8.30 | 35.38 | 6.72 | 4.30 | 15.97 | 1.35 |
| Alpheidae | 5.13 | 40.77 | 11.93 | 14.12 | 60.42 | 13.16 |
| Hippolytidae | 0.38 | 32.31 | 11.38 | 0.33 | 23.61 | 2.81 |
| Majidae | 1.97 | 4.62 | 0.28 | 4.45 | 9.72 | 0.67 |
| Portunidae | 17.06 | 23.85 | 2.96 | 5.00 | 14.58 | 0.60 |
| Xanthidae | 33.53 | 63.08 | 10.94 | 27.78 | 52.78 | 4.64 |
| Chordata | | | | | | |
| Teleostei | 21.25 | 56.92 | 7.43 | 24.87 | 43.75 | 2.31 |
| Batrachoididae | | | | | | |
| *Opsanus beta* | 18.94 | 48.46 | 5.85 | 19.87 | 25.00 | 1.19 |

**Table 7**

Breakdown into the most important prey groups of the mean dissimilarity between stomach contents (percent weight) of bonefish, *Albula vulpes* (480–699 mm FL), collected on the ocean side of the Florida Keys during January–March ($n$=39), April–June ($n$=43), and October–December ($n$=33). Prey groups are listed in order of decreasing contribution to the overall dissimilarity between the seasonal samples. Taxa proportionally more important in the diet of bonefish collected during January–March than during other seasons are shown with bold type. The low values of $\delta_i/SD(\delta_i)$ suggest that the data were variable and that no taxa were reliable discriminators of any particular season. Symbols are explained in the legend of Table 4.

| Species | $\delta_i$ | $\delta_i/SD(\delta_i)$ | $\delta_i$ % | Cum $\delta_i$ % |
|---|---|---|---|---|
| Jan–Mar vs. Apr–Jun | | | | |
| Xanthidae | 12.44 | 1.16 | 16.02 | 16.02 |
| **Alpheidae** | 11.77 | 1.28 | 15.16 | 31.18 |
| **Brachyura**[1] | 6.71 | 0.69 | 8.64 | 39.81 |
| *O. beta* | 5.41 | 0.62 | 6.97 | 46.78 |
| Stomatopoda | 5.18 | 0.64 | 6.67 | 53.45 |
| Jan–Mar vs. Oct–Dec | | | | |
| **Alpheidae** | 11.52 | 1.27 | 14.52 | 14.52 |
| Xanthidae | 10.99 | 0.99 | 13.86 | 28.38 |
| **Brachyura**[1] | 7.26 | 0.75 | 9.16 | 37.54 |
| Penaeidae | 4.97 | 0.76 | 6.27 | 43.81 |
| *O. beta* | 4.90 | 0.56 | 6.18 | 50.00 |

[1] Excluding xanthids, portunids, and majids.

fish smaller than 440 mm, although crabs of the genus *Portunus* were eaten in large numbers by one 435-mm bonefish.

There was evidence of a seasonal effect on diet, but small sample sizes during some seasons in each of the respective sampling areas reduced our ability to detect significant differences. Colton and Alevizon (1983) also found seasonal differences in feeding in Bahamian bonefish. Bivalves were eaten more during the summer by bonefish of all lengths, whereas small bonefish (<416 mm) ate more portunid crabs during the winter. They also noted habitat-related differences in bonefish feeding. Penaeid shrimp were eaten almost exclusively by bonefish caught over grassy bottom and not by those caught over sandy bottom. Bonefish caught over sandy bottom ate relatively more crabs and bivalves than did bonefish caught over grassy areas.

Warmke and Erdman (1963) examined the stomach contents of 56 bonefish ranging from 292 to 663 mm FL (reported as 0.75 to 10.25 pounds) from Puerto Rican waters and, like Colton and Alevizon (1983), found that mollusks were the most important prey. Stomachs of Puerto Rican bonefish contained 56% mollusks, 42% crustaceans, and 2% other prey types by volume. In contrast, we found that mollusks accounted for only about 7% of the diet of Keys bonefish by weight and that crustaceans accounted for about 70% of the diet. Teleosts were part of Warmke and Erdman's "other" classification and made up less than 2% of the diet in their study; in the Keys, teleosts made up over 20% of the diet by weight. Warmke and Erdman identified only mollusks to species. The most important mollusk they found was the bivalve *Codakia costata*, which occurred in 62% of the stomachs they examined. *Codakia orbicularis* and *C. orbiculata* occurred in stomachs from Keys bonefish but made up less than 1% of the diet by numbers or weight. *Codakia costata* was not found in Keys bonefish stomachs and was not reported to occur in Florida Bay by Turney and Perkins (1972).

There were slight but significant differences between the diets of bonefish from Florida Bay and those from the ocean side of the Keys. These differences may reflect differences in prey availability in the two areas, but overall the dominant prey eaten by bonefish was the same in the two areas. The fauna of Florida Bay has been characterized as Gulf-Carolinian in nature, whereas that of the Keys ocean side is Antillean (Sogard et al., 1987; Holmquist et al.,

**Table 8**

Breakdown into the most important prey groups of the mean dissimilarity between stomach contents (percent number) of bonefish, *Albula vulpes* (480–699 mm FL), caught on the ocean side of the Florida Keys ($n$=39) and throw-trap samples ($n$=54) from the ocean side of the Florida Keys. Prey groups are listed in order of decreasing contribution to the overall dissimilarity between the two samples. Taxa proportionally more important in the diet of bonefish than suggested by their proportional abundance in throw-trap samples are shown with bold type. The low values of $\delta_i/SD(\delta_i)$ suggest that the data were variable and that no taxa were reliable discriminators of either sample source. Symbols are explained in the legend of Table 4.

| Species | $\delta_i$ | $\delta_i/SD(\delta_i)$ | $\delta_i$ % | Cum $\delta_i$ % |
|---|---|---|---|---|
| **Alpheidae** | 10.58 | 1.31 | 13.87 | 13.87 |
| **Xanthidae** | 7.89 | 1.13 | 10.35 | 24.23 |
| *Periclimenes americanus* | 6.83 | 0.93 | 8.96 | 33.19 |
| *Thor* spp. | 6.11 | 0.97 | 8.02 | 41.20 |
| ***P. duorarum*** | 5.95 | 0.88 | 7.81 | 49.01 |
| ***O. beta*** | 4.61 | 0.80 | 6.05 | 55.06 |

**Table 9**

Breakdown into the most important prey groups of the mean dissimilarity between stomach contents (percent number) of bonefish, *Albula vulpes* (480-699 mm FL), caught in Florida Bay ($n$=45) and throw-trap samples ($n$=30) from Florida Bay (Matheson et al.[1]). Prey groups are listed in order of decreasing contribution to the overall dissimilarity between the two samples. Taxa that are likely to be reliable discriminators of the two samples are indicated by ** in the $\delta_i/SD(\delta_i)$ column. Taxa proportionally more important in the diet of bonefish than suggested by their proportional abundance in throw-trap samples are shown with bold type. Symbols are explained in the legend of Table 4.

| Species | $\delta_i$ | $\delta_i/SD(\delta_i)$ | $\delta_i$ % | Cum $\delta_i$ % |
|---|---|---|---|---|
| *Thor* spp. | 18.13 | 1.97** | 23.03 | 23.03 |
| **Xanthidae** | 9.63 | 1.17 | 12.24 | 35.27 |
| **Alpheidae** | 7.61 | 1.23 | 9.67 | 44.94 |
| ***O. beta*** | 7.51 | 1.15 | 9.55 | 54.49 |
| ***P. duorarum*** | 5.17 | 0.81 | 6.57 | 61.06 |
| *Hippolyte zostericola* | 5.00 | 1.65** | 6.36 | 67.42 |
| *Periclimenes americanus* | 4.55 | 1.85** | 5.78 | 73.20 |
| ***Callinectes* spp.** | 3.23 | 0.48 | 4.10 | 77.30 |
| *Gobiosoma robustum* | 3.03 | 1.98** | 3.85 | 81.15 |

1989a, 1989b). Our sampling effort was over a large and diverse area, and this limited our ability to resolve area-specific differences in bonefish diet. Some Florida Bay areas that we sampled were near passes leading to ocean-side flats and may have more closely resembled ocean-side areas than some of the more remote areas in Florida Bay where we occassionally caught bonefish. Larger sample sizes, more intensive sampling of specific areas along with site-specific descriptions of habitat types, and sampling of prey availability concurrent with bonefish collections are needed to better describe spatial variation in the diet of Keys bonefish.

Comparisons of the stomach contents of bonefish collected in Florida Bay and ocean-side areas as well as seasonal comparisons were complicated by the variable monthly sample sizes from the two areas. We excluded over half of the bonefish in our sample from our area comparisons because seasonal sample sizes from the two areas were greatly unequal. The variable sample sizes from the two areas reflect general seasonal trends in bonefish availability in the two areas. Bonefish are typically most abundant in Florida Bay during summer and fall. Winter cold fronts tend to reduce Florida Bay temperatures more than ocean-side temperatures (Hudson et al., 1976; Roberts et al., 1982; Chiappone, 1996), and many productive summer–fall fishing areas in Florida Bay rarely hold bonefish during winter and spring because bonefish move to ocean-side areas with more moderate temperatures and closer proximity to deep water. Thus, most of our Florida Bay bonefish were captured during summer and fall, and most ocean-side bonefish were caught during winter and spring.

Seagrass die-offs have recently been documented in Florida Bay (Robblee et al., 1991; Carlson et al., 1994; Durako, 1994; Butler et al., 1995). Anecdotal evidence suggests that changes in the Everglades ecosystem have caused a decline in the quality of fishing in Florida Bay and the waters of the Florida Keys (Chiappone and Sulka, 1996). If changes in the benthic epifauna and infauna have resulted from the seagrass die-off, these changes could potentially affect feeding and occurrence of bonefish in Florida Bay. Data on the species composition and abundance of epifaunal crustaceans and fishes collected subsequent to the sea grass die-off and the studies of Sogard et al. (1987, 1989) and Holmquist et al. (1989a, 1989b) prior to the seagrass die-off suggest little evidence of declines in populations of important bonefish prey species (Matheson et al.[1]). One significant change reported by Matheson et al.[1] was an increase in the abundance of *O. beta* in some areas of Florida Bay since the 1980s. Whether the increased abundance of *O. beta* compared to that found in previous studies accounts for its greater prominence in stomachs of the bonefish we sampled is unknown.

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## Literature cited

**Bray, J. R., and J. T. Curtis.**
**1957.** An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27:325–349.

**Bruger, G. E.**
**1974.** Age, growth, food habits, and reproduction of bonefish, *Albula vulpes*, in South Florida waters. Fla. Mar. Res. Publ. 3, 20 p.

**Butler, M. J., IV, J. H. Hunt, W. F. Herrnkind, M. J. Childress, R. Bertelsen, W. Sharp, T. Matthews, J. M. Field, and H. G. Marshall.**
**1995.** Cascading disturbances in Florida Bay, USA: cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. Mar. Ecol. Prog. Ser. 129:119–125.

**Carlson, P. J. Jr., L. A. Yarbro, and T. R. Barber.**
**1994.** Relationship of sediment sulfide to mortality of *Thalassia testudinum* in Florida Bay. Bull. Mar. Sci. 54:733–746.

**Chiappone, M.**
**1996.** Oceanography and shallow-water processes of the Florida Keys and Florida Bay. Site characterization for the Florida Keys National Marine Sanctuary and environs, vol. 2. Farley Court Publ., Zenda, WI, 86 p.

**Chiappone, M., and R. Sulka.**
**1996.** Fishes and fisheries. Site characterization for the Florida Keys National Marine Sanctuary and environs, vol. 2. Farley Court Publ., Zenda, WI, 149 p.

**Clarke, K. R.**
**1993.** Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18:117–143.

**Clarke, K. R., and R. M. Warwick.**
**1994.** Change in marine communities: an approach to statistical analysis and interpretation. Natl. Environ. Res. Council, U.K., 144 p.

**Colton, D. E., and W. S. Alevizon.**
**1983.** Feeding ecology of bonefish in Bahamian waters. Trans. Am. Fish. Soc. 112:178–184.

**Crabtree, R. E., C. W. Harnden, D. Snodgrass, and C. Stevens.**
**1996.** Age, growth, and mortality of bonefish, *Albula vulpes*, from the waters of the Florida Keys. Fish. Bull. 94:442–451.

**Crabtree, R. E., D. Snodgrass, and C. W. Harnden.**
**1997.** Maturation and reproductive seasonality in bonefish, *Albula vulpes*, from the waters of the Florida Keys. Fish. Bull. 95:456–465.

**Durako, M. J.**
**1994.** Seagrass die-off in Florida Bay (USA): changes in shoot demographic characteristics and population dynamics in *Thalassia testudinum*. Mar. Ecol. Prog. Ser. 110:59–66.

**Holmquist, J. G., G. V. N. Powell, and S. M. Sogard.**
**1989a.** Decapod and stomatopod communities of seagrass-covered mud banks in Florida Bay: inter- and intra-bank heterogeneity with special reference to isolated subenvironments. Bull. Mar. Sci. 44:251–262.
**1989b.** Decapod and stomatopod assemblages on a system of seagrass-covered mud banks in Florida Bay. Mar. Biol. 100:473–483.

**Hudson, J. H., E. A. Shinn, R. B. Halley, and B. Lidz.**
**1976.** Sclerochronology, a tool for interpreting past environments. Geology 4:361–364.

**Robblee, M. B., T. R. Barber, P. R. Carlson, M. J. Durako, J. W. Fourqurean, L. K. Muehlstein, D. Porter, L. A. Yarbro, R. T. Zieman, and J. C. Zieman.**
**1991.** Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay (USA). Mar. Ecol. Prog. Ser. 71:297–299.

**Roberts, H. H., L. J. Rouse, Jr., N. D. Walker, and J. H. Hudson.**
**1982.** Cold-water stress in Florida Bay and the Bahamas: a product of winter cold-air outbreaks. J. Sediment. Petrol. 52:145–155.

**Sogard, S. M., G. V. N. Powell, and J. G. Holmquist.**
**1987.** Epibenthic fish communities on Florida Bay banks: relations with physical parameters and seagrass cover. Mar. Ecol. Prog. Ser. 40:25–39.
**1989.** Spatial distribution and trends in abundance of fishes residing in seagrass meadows on Florida Bay mud-banks. Bull. Mar. Sci. 44:179–199.

**Turney, W. J., and B. F. Perkins.**
**1972.** Molluscan distribution in Florida Bay: sedimentia III. Rosenstiel School of Marine and Atmospheric Science, Miami, FL. 37 p.

**Warmke, G. L., and D. S. Erdman.**
**1963.** Records of marine mollusks eaten by bonefish in Puerto Rican waters. The Nautilus 76:115–120.

**Zieman, J. C., J. W. Fourqurean, and R. L. Iverson.**
**1989.** Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. Bull. Mar. Sci. 44:292–311.

# Population structure in greater amberjack, *Seriola dumerili*, from the Gulf of Mexico and the western Atlantic Ocean*

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**Abstract**–Variation in restriction sites of mitochondrial (mt)DNA was examined from 444 greater amberjack (*Seriola dumerili*) sampled from 11 offshore localities in the northern Gulf of Mexico (Gulf) and along the U.S. southeast Atlantic coast (Atlantic). A total of 49 mtDNA haplotypes (genotypes) were detected. Percent nucleotide sequence divergence among the haplotypes ranged from 0.156 to 2.623 (mean ±SE=0.980 ±0.015). Nucleon diversity within samples ranged from 0.845 to 0.906, and intrapopulational mtDNA diversities ranged (mean ±SD) from 0.483 ±0.370 to 0.619 ±0.419. The latter did not differ significantly from one another. Homogeneity tests of mtDNA haplotype frequencies, $F_{ST}$ values, analysis of molecular variance (AMOVA), and comparisons of pairwise $\Phi_{ST}$ distances were consistent with the hypothesis that (at least) two subpopulations (stocks) of greater amberjack exist in U.S. waters: one in the northern Gulf and one along the U.S. Atlantic coast. The latter subpopulation includes individuals from the Florida Keys. There was no evidence of phylogeographic structure among mtDNA haplotypes or among sample localities, suggesting either that restricted gene flow between the subpopulations is fairly recent or that gene flow between the two is relatively infrequent. No significant spatial autocorrelations of mtDNA haplotypes was found among samples of greater amberjack from the Gulf, indicating continuous gene flow across the northern Gulf. Long-term effective (female) population sizes of both subpopulations were estimated to be in the range of 90,000–95,000 individuals. The estimates were commensurate with estimates in other, economically important marine fish. Based on suggested differences in mtDNA evolutionary rates between homeothermic and poikilothermic vertebrates, the effective (female) population sizes of both stocks of greater amberjack could be in the range of 500,000 to 1,000,000 individuals.

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Fish. Bull. 96:767–778 (1998).

Greater amberjack, *Seriola dumerili*, constitute an increasingly important component of the U.S. Gulf and Atlantic commercial and recreational fisheries. Commercial landings in the Atlantic rose over twentyfold between 1981 and 1991, from under 100,000 pounds annually to nearly two million pounds (Cummings-Parrack[1]). Commercial landings in the Gulf rose similarly, reaching a peak of nearly 2.5 million pounds in 1988 that was then followed by a >50% decline in 1990 and a further decline in 1991 (McClellan and Cummings[2]). An increase in commercial landings in 1993 was followed by declines in both 1994 and 1995 (McClellan and Cummings[2]). The commercial interest in greater amberjack appears to be driven by: 1) consumer acceptance of greater amberjack as an edible fish; 2) replacement of red drum in the blackened fish market; and 3) effort displacement of king mackerel and reef-fish fishermen, especially off the coasts of Florida (Cummings-Parrack[1]). Recreational landings are smaller in both the Gulf and Atlantic and appear to have declined in recent years (Cummings-Parrack[1]; McClellan and Cummings[2]). Problems that confound landing statistics and fishery analysis of the greater amberjack resource include species misidentification and the possibility that reported landings may be small in proportion to total removals (Cummings-Parrack[1]). Current management of the greater amberjack resource in U.S. waters is based on a two-stock (management unit) model. One stock resides along the U.S. southeastern coast and includes all fishing areas north of Cape Hatteras, NC (35°00'N) southward to the Dry Tortugas and the Florida Keys (24°35'N); the other includes fishing grounds in the Gulf of Mexico off the Dry Tortugas and Florida Keys north and then westward to the U. S. Mexico border (26°00'N)

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[1] Cummings-Parrack, N. 1993. The exploitation status of the Atlantic amberjack fisheries through 1991. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-92/93-30, Miami, FL, 98 p.

[2] McClellan, D. B., and N. J. Cummings. 1996. Stock assessment of Gulf of Mexico greater amberjack through 1995. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-96/97-03, Miami, FL, 69 p.

(Cummings and McClellan[3]). Movement patterns inferred from mark-and-release experiments carried out between 1959 and 1994 (Cummings and McClellan[3]) are consistent with the two-stock hypothesis. In brief, over 1400 recaptures from approximately 14,000 releases revealed cyclical tag-return patterns that suggested resident stocks or subpopulations of greater amberjack along both the eastern coast of Florida and the northern Gulf. Exchange rates between the two stocks was estimated as approximately 1.5% by Cummings and McClellan[3] although, as noted by these authors, the rate estimates were not adjusted for fishing pressure or for potential biases due to mortality, tag shedding, lack of reporting, and fishing effort. It also was clear from a few tag returns that greater amberjack can migrate considerable distances, e.g. from near Charleston, South Carolina, to Texas or from northwest Florida to Virginia (Cummings and McClellan[3]).

Biological information on greater amberjack is limited to studies reported in Berry and Burch (1978), Shipp (1986), Manooch (1988), and GMFMC (1989). Thompson et al.[4] presented data on greater amberjack age, growth, and reproduction, and Cummings-Parrack[1] and McClellan and Cummings[2] summarized most of the available information on landings and other fishery statistics. Direct or indirect information on genetic stock structure is even more limited. Johnson[5] carried out a pilot study of nuclear-gene (allozyme) variation among 225 greater amberjack sampled from the Atlantic ($n$=60), eastern Gulf ($n$=84), and western Gulf ($n$=81). Of 72 putative loci examined, only one polymorphic (and noninformative) system was found. On the surface, these data do not support the concept of separate stocks. However, genetic homogeneity (*sensu stricto*) does not unequivocally establish the existence of a single breeding population (stock), but rather is simply consistent with the hypothesis that samples are drawn from a population with the same parametric allele frequencies. In addition, the almost total absence of variation effectively precluded rigorous testing of the null hypothesis (i.e. the interpretation of genetic homogeneity among samples is potentially compromised by virtue of the absence of significantly variable nuclear-gene loci).

Alternatively, the apparent absence of genetic variation raises considerable concern about the effective size of greater amberjack populations. Compared with other marine finfish (Smith and Fujio, 1980; Waples, 1987; Bohlmeyer and Gold, 1991), levels of nuclear-gene variation in greater amberjack (as reported by Johnson[5]) are low. Richardson and Gold (1993) examined mitochondrial (mt)DNA variation among 59 greater amberjack sampled primarily from the west coast of Florida. Levels of mtDNA variation in greater amberjack were low in comparison with red drum and several clupeid species (e.g. Atlantic menhaden), but higher than those found in black drum, red snapper, and red grouper (Camper et al., 1993; Gold et al., 1993; Richardson and Gold, 1993). Estimates of long-term, effective female population size (computed directly from levels of mtDNA variation) paralleled levels of mtDNA variation, suggesting that effective (female) population sizes of Gulf greater amberjack were not atypically low.

Concerns regarding greater amberjack fisheries in the Gulf and Atlantic include the following: 1) presumed decreases in average individual size in both Gulf and Atlantic fisheries; 2) apparent declines in size of the presumed Atlantic stock; 3) a trend of declining yield in both commercial and headboat fisheries in the Gulf; and 4) apparent highly erratic recruitment where success of individual year classes is quite variable (Cummings-Parrack[1]; Cummings and McClellan[3]). These concerns have intensified as the economic importance of greater amberjack has grown (GMFMC, 1989; Cummings and McClellan[3]). In this study, we employed variation in restriction sites in mitochondrial (mt)DNA of greater amberjack to determine if significant population structure (separate genetic stocks) occurs in U.S. waters, i.e. in the northern Gulf of Mexico and along the U.S. southeastern Atlantic coast. The rationale for this study is the need for accurate geographic definition when conducting stock assessments (Hilborn, 1985; Sinclair et al., 1985), in this case for greater amberjack in U.S. waters.

## Materials and methods

Appropriate tissues (heart and white muscle) were obtained from a total of 444 greater amberjack sampled from 11 offshore localities in U.S. waters (Table 1; Fig. 1). With exception of a sample of seven individuals from near Gulfport, MS, sample sizes

[3] Cummings, N. J., and D. B. McClellan. 1996. Movement patterns and stock interchange of greater amberjack *Seriola dumerili*, in the southeastern U.S. Miami Laboratory, SE Fisheries Sci. Center, Natl. Mar. Fish. Serv., Cont. MIA-95/96-14, Miami, FL, 24 p.

[4] Thompson, B. A., C. A. Wilson, J. H. Render, H. Beasley, and C. Cauthron. 1992. Age, growth, and reproductive biology of greater amberjack and cobia from Louisiana waters. Final Rep., Marfin Prog., U.S. Dep. Comm., Coop. Agreement NA90AA-H-MF722, 77 p.

[5] Johnson, A. G. 1990. Progress report: electrophoretic examination of greater amberjack (*Seriola dumerili*). Panama City Laboratory, SE Fish. Sci. Center, Natl. Mar. Fish. Serv., Panama City, FL, 34 p.

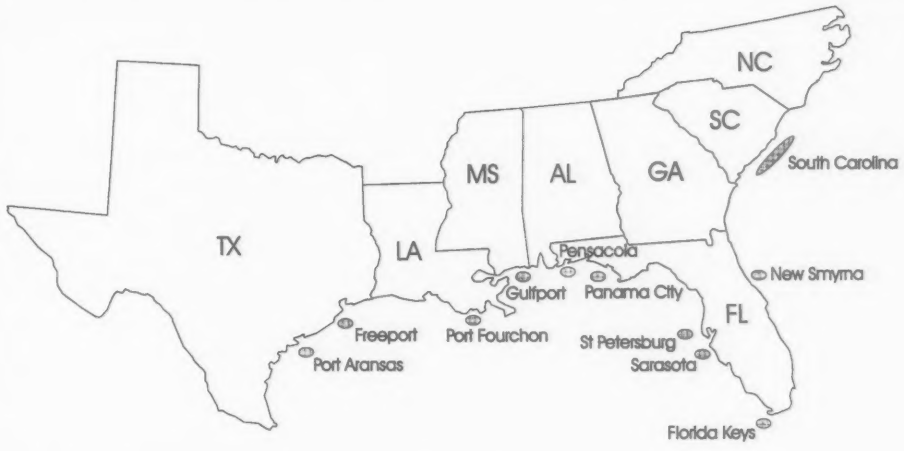


**Figure 1**

Dockside localities for greater amberjack, *Seriola dumerili*, in present study: NC = North Carolina; SC = South Carolina; GA = Georgia; FL = Florida; AL = Alabama; MS = Mississippi; LA = Louisiana; and TX = Texas.

ranged from 24 to 58 individuals. Individuals were sampled variously from charter boats, headboats, and commercial fishing boats, and from catches of recreational fishers at tournaments. Tissues were removed from each specimen, quickly frozen in liquid nitrogen, and transported to Texas A&M University where they were stored at –80°C in an ultracold freezer.

Methods (including DNA extraction, precipitation, and storage) used to assay restriction-enzyme fragment patterns of mtDNA molecules of individual fish followed those described in Gold and Richardson (1991). Sixteen, type-II restriction-endonuclease enzymes were used to digest 1.0–1.5 µg of DNA in 40 µL reactions according to manufacturer's specifications. Enzymes used were *Apa*LI, *Apa*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco*I, *Pst*I, *Pvu*II, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Stu*I, and *Xba*I. Methods of DNA digestion, agarose electrophoresis, transfer to nylon filters (after Southern, 1975), hybridization, and autoradiography also followed those in Gold and Richardson (1991). Hybridization employed a 12.5 kilobase (kb) fragment of greater amberjack mtDNA cloned into lambda bacteriophage (Richardson and Gold, 1993). Bacteriophage lambda DNA digested with restriction enzyme *Hin*dIII was employed as a molecular weight marker on each gel. MtDNA fragments produced by single digestion of greater amberjack mtDNA were sized by fitting migration distances to a least-squares regression of lambda DNA-*Hin*dIII fragment migration distances. Single digestion mtDNA-fragment patterns were used to generate composite digestion patterns (mtDNA haplotypes).

**Table 1**

Sample localities of greater amberjack (*Seriola dumerili*).

| Locality[1] | Number of individuals |
|---|---|
| Port Aransas, Texas (PA) | 58 |
| Freeport, Texas (FP) | 44 |
| Port Fourchon, Louisiana (PF) | 43 |
| Gulfport, Mississippi (GP) | 7 |
| Pensacola, Florida (PN)[2] | 24 |
| Panama City, Florida (PC) | 50 |
| St. Petersburg, Florida (SP) | 42 |
| Sarasota, Florida (SR)[2] | 32 |
| Florida Keys (FK) | 55 |
| New Smyrna Beach, Florida (NS) | 53 |
| South Carolina (SC) | 36 |
| Total | 444 |

[1] Localities represent dock sites where individuals sampled offshore were off-loaded. Individuals from the Florida Keys were off-loaded at Islamorada; individuals from South Carolina were off-loaded at several localities.
[2] Previously examined by Richardson and Gold (1993).

Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Genotypic (nucleon) diversity within sample localities was calculated following Nei and Tajima (1981) and was based on the total number of mtDNA haplotypes identified within a local-

ity. This value represents the probability that any two individuals drawn at random will differ in mtDNA haplotype. Intrapopulational nucleotide sequence diversity within sample localities also was estimated after Nei and Tajima (1981). This value represents the average nucleotide sequence difference between any two individuals drawn at random from a given sample or locality.

Homogeneity testing of mtDNA haplotype frequencies among sample localities was carried out by using 1) a randomization (bootstrap) procedure developed by Roff and Bentzen (1989), 2) a log-likelihood ($G$) test (Sokal and Rohlf, 1969), and 3) $V$ tests that employed arcsine, square-root-transformed haplotype frequencies (DeSalle et al., 1987). Significance levels for multiple tests were adjusted after Rice (1989). $F_{ST}$ values, a measure of the variance in mtDNA haplotype frequencies, were calculated after Weir and Cockerham (1984) by using algorithms described in Weir (1990). Significance of $F_{ST}$ values was tested by the randomization procedure in version 1.4 of the analysis of molecular variance (AMOVA) program of Excoffier et al. (1992). The latter program (AMOVA) was used primarily to examine the distribution of variance in mtDNA haplotypes. AMOVA analysis generates estimates of (genetic) variance components and a set of hierarchical $F$-statistic analogs ($\Phi$ statistics) that are tested for significance through random permutation methods. The permutation approach avoids the parametric assumptions of normality and independence normally not met by molecular distance measures (Excoffier et al., 1992). Sample localities were nested into regional groupings, i.e. Gulf and Atlantic, that were input into AMOVA. In one AMOVA run, the sample from the Florida Keys was included with the Gulf group, whereas in a second run it was included in the Atlantic group.

Restriction-site presence and absence matrices for individual mtDNA haplotypes were constructed with the GENERATE program in REAP by inferring restriction site gains and losses for each enzyme. Maximum-parsimony analysis of restriction-site presence and absence matrices representing all haplotypes employed MULPARS and CONTREE options in version 3.1 of the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1991). Autapomorphic and symplesiomorphic characters were removed prior to PAUP runs. Nucleotide-sequence divergence values among sample localities (interpopulational divergence) were generated following Nei and Tajima (1981) and Nei and Miller (1990). MtDNA-based similarity among sample localities was assessed with the neighbor joining method (Saitou and Nei, 1987) and employed the NEIGHBOR program in Phylogenetic Inference Package (PHYLIP), version 3.4 of Felsenstein (1989).

The spatial distribution of mtDNA haplotypes was investigated by means of spatial autocorrelation analysis (SAAP; Wartenberg, 1989). This analysis determines whether haplotype frequencies at any sample locality are independent of haplotype frequencies at neighboring sample localities. Correlograms that plot autocorrelation coefficients (Moran's $I$ values) as a function of geographic distance between pairs of localities were used to summarize patterns of geographic variation of haplotype frequencies. To minimize "noise" generated by low-frequency haplotypes, Moran's $I$ values were calculated only for haplotypes occurring in eight or more individuals (10 haplotypes total). These included haplotypes 1–4, 6, 11, 13, 23, 37, and 48.

## Results

Single digestions of mtDNA molecules from the 444 individuals surveyed produced variable fragment patterns for the 16 restriction enzymes. The majority of fragment patterns observed are given in Appendix Table A2 of Richardson and Gold (1993). New fragment patterns revealed in our study are as follows (fragment sizes in base pairs; asterisks represent fragments assumed to exist but not covered by the mtDNA probe): *Eco*RI, pattern C (8700, 5025, 3175*); *Hpa*I, pattern C (8800, 5550, 2550); *Nco*I, pattern C (11050, 5850); *Sst*I, pattern C (11700, 3450, 1750); *Sca*I, pattern C (7500, 3600, 3100, 2700) and pattern D (10000, 3600, 3300); *Sma*I, pattern D (10700, 4250, 1500, 450); *Spe*I pattern D (7900, 5800, 1200, 1200*, 800); *Ssp*I, pattern C (6600, 6200, 4100); and *Xba*I, pattern D (7300, 5000, 4600) and pattern E (7300, 4600, 2600, 2275, 125*). The mean genome size of all apparently complete digestion patterns was 16.9 ±0.2 kilobase pairs. No evidence of mtDNA size variation or heteroplasmy was observed. All fragment patterns for each restriction enzyme were consistent with the hypothesis of single nucleotide substitutions. A total of 72 unique restriction sites was inferred from the digestion patterns.

A total of 49 mtDNA haplotypes was identified among the 444 individuals surveyed (Table 2). Four haplotypes (8, 14, 16, and 18) are not listed in Table 2; these were listed in Richardson and Gold (1993) and were identified by three restriction enzymes (*Cla*I, *Nsi*I, and *Pvu*I) not employed in our study. Four haplotypes (1, 4, 6, and 13) were abundant, occurring in 77, 91, 54, and 70 individuals, respectively. Two haplotypes (2 and 3) occurred in 20 and 22 individuals, respectively, whereas the remainder occurred in 10 or fewer individuals. Twenty haplotypes were found in only one individual each. Estimates of

**Table 2**

Spatial distribution of mitochondrial (mt)DNA haplotypes of greater amberjack (*Seriola dumerili*). Letters (from left to right) are digestion patterns for *Apa*LI, *Apa*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco*I, *Pst*I, *Pvu*II, *Sca*I, *Sma*I, *Spe*I, *Ssp*I, *Sst*I, *Stu*I, and *Xba*I.

| Haplotype no. | Composite mtDNA genotype | Samples | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PA | FP | PF | GP | PN | PC | SP | SR | FK | NS | SC |
| 1 | AAAAAAAAAAAAAAAA | 7 | 10 | 8 | 2 | 5 | 8 | 12 | 7 | 4 | 8 | 6 |
| 2 | AAAABBAAAAAAAAAA | 4 | 4 | 3 | — | 1 | 2 | 3 | — | 3 | 2 | 1 |
| 3 | AAAAABAAAAAAAABA | 3 | 2 | 3 | — | 3 | 1 | 1 | — | 4 | 4 | 1 |
| 4 | AAAAABAAAAAAAAAA | 15 | 9 | 10 | 1 | 5 | 11 | 4 | 10 | 10 | 9 | 7 |
| 5 | AABBABAAAABAABAA | — | 1 | — | — | 3 | — | — | 1 | — | — | — |
| 6 | AAAAAAAAAAAAAABA | 8 | 4 | 2 | 1 | — | 4 | 4 | 5 | 10 | 6 | 10 |
| 7 | AAAAAAAAAAABAAAA | — | — | — | — | — | — | — | 1 | — | — | — |
| 9 | BABAAAAAAACAAABA | — | — | — | 1 | — | — | — | 1 | 1 | — | — |
| 10 | BAAAABBBAAAAAAAA | — | — | 1 | — | — | 2 | — | 1 | — | — | — |
| 11 | BABAAAAAAAAAAABA | 1 | 1 | 2 | — | — | 1 | 1 | 1 | — | 1 | 1 |
| 12 | AAAAAAAAABACAAAA | — | — | — | — | — | — | — | 1 | — | 2 | — |
| 13 | AAAAAAAAABABAAAA | 7 | 4 | 4 | 2 | 1 | 12 | 7 | 2 | 10 | 15 | 6 |
| 15 | AAAAAAAAAAAABABA | — | — | 1 | — | — | 1 | — | 1 | — | — | — |
| 17 | AAAAABAABAAAAAAC | — | — | — | — | 1 | — | — | — | — | — | — |
| 19 | AAAAABACAAAAAAAA | — | — | — | — | 1 | — | — | — | — | — | — |
| 20 | AAAAAAAAAAAAAABB | — | — | — | — | 1 | — | — | — | — | — | — |
| 21 | AAAAABABAAAAAAAA | — | 1 | — | — | 1 | 1 | — | — | — | — | — |
| 22 | ABAACAAACBAAAABA | — | — | — | — | 1 | — | — | — | — | — | — |
| 23 | AABAABAAAAAAAAAA | 3 | 1 | 2 | — | 1 | 1 | — | — | — | — | — |
| 24 | BAAAAAAAAAAABABA | — | 1 | — | — | — | — | — | — | — | — | — |
| 25 | AAAAAAAAACABCAAA | — | — | — | — | — | — | — | — | 2 | — | — |
| 26 | BAAABAAAAAAAAAAA | — | 1 | — | — | — | — | — | — | — | — | — |
| 27 | ABAACAAACAAAACBA | 1 | — | — | — | — | — | — | — | — | — | — |
| 28 | AAAAAAAAABAAAAAA | — | 2 | — | — | — | — | 1 | — | — | — | — |
| 29 | AAAACAAAAAAAAABA | — | 1 | — | — | — | — | — | — | — | — | — |
| 30 | AAAABBAAAAAAACAA | — | — | 1 | — | — | — | — | — | — | — | — |
| 31 | AAAAABBAAAAAAAAA | — | 1 | — | — | — | — | — | — | — | — | — |
| 32 | AAAACAAAADDDAABD | — | 1 | 1 | — | — | — | — | — | — | — | — |
| 33 | AAABAAAAAAAAAAAA | 1 | — | — | — | — | 1 | — | — | — | — | — |
| 34 | AACAAAAAABABAAAA | 2 | — | — | — | — | — | — | — | — | — | — |
| 35 | AAAAABAAAAAAAACA | 1 | — | — | — | — | — | 1 | 1 | 1 | — | — |
| 36 | BABAABAAAAAAAABA | 1 | 1 | — | — | — | — | — | — | — | — | — |
| 37 | ABAACAAACAAAAABA | 1 | — | 2 | — | — | — | 2 | — | 4 | 1 | — |
| 38 | AAAAAAAAACABAAAA | 1 | — | — | — | — | 1 | — | — | — | 1 | — |
| 39 | AABACCAAADDDAABD | — | — | — | — | — | — | — | — | — | 1 | — |
| 40 | AAAAABAAAAAACAAA | — | — | — | — | — | — | 1 | — | — | 1 | — |
| 41 | ABAAAAAAABABAAAA | — | — | — | — | — | — | — | — | — | 1 | — |
| 42 | AAAAABAAAAAABAAA | — | — | — | — | — | — | — | — | 1 | — | — |
| 43 | AAAAABAAAAAAAAAE | — | — | — | — | — | 1 | — | — | — | — | — |
| 44 | AAAAABCAAAAAAABA | — | — | 1 | — | — | 1 | — | — | — | — | — |
| 45 | AAAAABCAAAAAAAAA | — | — | 1 | — | — | 1 | — | — | — | — | — |
| 46 | AAAACCAAADDDCABD | 1 | — | — | — | — | 1 | — | — | — | — | — |
| 47 | AAAAABAAAABAABAA | — | — | — | — | — | — | — | — | 1 | — | — |
| 48 | AAAAABAAAAAAACAA | 1 | 2 | — | — | — | — | 2 | — | 2 | 1 | — |
| 49 | AAAAABAAABABAAAA | — | — | 1 | — | — | — | — | — | — | — | — |
| 50 | AAAAABAAABAAAAAA | — | — | — | — | — | — | 1 | — | — | — | — |
| 51 | AAAACAAACAAAAABA | — | — | — | — | — | — | 1 | — | — | — | 1 |
| 52 | AABAABAAAABAABAA | — | — | — | — | — | — | — | — | — | — | 1 |
| 53 | AAAACAAAAAADAAAD | — | — | — | — | — | — | 1 | — | — | — | — |

the percentage nucleotide-sequence divergence among the 49 haplotypes ranged from 0.156 to 2.623 (mean ±SE=0.980 ±0.015). MtDNA nucleon diversity was 0.905, and intrapopulational nucleotide sequence diversity was 0.548 ±0.412 (mean ±SD). The latter estimates were based on all 444 individuals surveyed. Nucleon diversity within samples (Table 3) ranged from 0.845 in the sample from Sarasota, Florida, to

**Table 3**

Mitochondrial (mt)DNA nucleon and intrapopulational nucleotide sequence diversities among samples of greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and Atlantic Ocean

| Locality | Number of individuals | Number of haplotypes | Nucleon diversity | Nucleotide sequence diversity (±SD)[1] |
|---|---|---|---|---|
| Port Aransas, TX | 58 | 17 | 0.886 | 0.561 ± 0.443 |
| Freeport, TX | 44 | 18 | 0.899 | 0.515 ± 0.387 |
| Port Fourchon, LA | 43 | 16 | 0.901 | 0.579 ± 0.435 |
| Gulfport, MS | 7 | 5 | 0.905 | 0.587 ± 0.415 |
| Pensacola, FL | 24 | 12 | 0.906 | 0.601 ± 0.449 |
| Panama City, FL | 50 | 17 | 0.872 | 0.525 ± 0.415 |
| St. Petersburg, FL | 42 | 15 | 0.879 | 0.519 ± 0.395 |
| Sarasota, FL | 32 | 12 | 0.845 | 0.483 ± 0.370 |
| Florida Keys | 55 | 14 | 0.893 | 0.619 ± 0.419 |
| New Smyrna, FL | 53 | 14 | 0.861 | 0.537 ± 0.427 |
| South Carolina | 36 | 10 | 0.846 | 0.502 ± 0.342 |
| Total | 444 | 49 | 0.905 | 0.548 ± 0.412 |

[1] In per cent.

0.906 in the sample from Pensacola, Florida. Intrapopulational nucleotide sequence diversity within samples (Table 3) ranged (mean ±SD) from 0.483 ±0.370 in the sample from Sarasota, Florida, to 0.619 ±0.419 in the sample from the Florida Keys. The latter values are all within one standard error (estimated from bootstrap analysis) of one another, indicating that levels of mtDNA variation are essentially identical throughout the geographic area surveyed. Levels of mtDNA variability, as measured by nucleon diversity and intrapopulational nucleotide sequence diversity, are commensurate with those estimated for several other marine fish of commercial or recreational value (Gold et al., 1993).

Results of bootstrap analyses and log-likelihood tests of spatial homogeneity in mtDNA haplotype frequencies are shown in Table 4. Tests were carried out 1) among all sample localities, 2) among samples from the Gulf, and 3) between defined groups where samples were pooled. In the last category, defined groups were based on region, i.e. Gulf and Atlantic. Gulf samples included the following: Port Aransas, TX; Freeport, TX; Port Fourchon, LA; Gulfport, MS; Pensacola, FL; Panama City, FL; and Sarasota, FL. Atlantic samples included New Smyrna, FL, and South Carolina. Two sets of pooled comparisons were carried out: in one, the sample from the Florida Keys was included with Gulf samples; in the other, the sample from the Florida Keys was included with Atlantic samples. We used this approach because the sample from the Florida Keys is located on the geographic boundary between the two putative stocks of greater amberjack (Cummings and McClellan[3]),

**Table 4**

Tests for spatial homogeneity in mtDNA haplotype frequencies among greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and U.S. southeastern Atlantic. $F_{ST}$ is a measure of variance in haplotype frequencies. Number of samples is in parentheses.

| Test group | Number of haplotypes | Homogeneity tests[1] $P_{RB}$ | $P_G$ | $F_{ST}$ |
|---|---|---|---|---|
| All samples (11) | 49 | 0.158 | >0.050 | 0.005 |
| Gulf samples (8) | 45 | 0.250 | >0.050 | 0.003 |
| Pooled comparisons | | | | |
| Gulf + Florida Keys vs. Atlantic (2) | 49 | 0.627 | >0.050 | 0.004 |
| Atlantic + Florida Keys vs. Gulf (2) | 49 | 0.042 | ~0.002 | 0.009[2] |

[1] $P_{RB}$ is probability from randomization (bootstrap) approach of Roff and Bentzen (1989); $P_G$ is probability from log-likelihood ($G$) test (Sokal and Rohlf, 1969).
[2] Value differs significantly ($P$=0.007) from 0.00; all other $F_{ST}$ values are nonsignificant.

and we could not place *a priori* the sample from the Florida Keys into either stock before testing the two-stock hypothesis. Significant heterogeneity was found only in the pooled comparison of samples from the Atlantic and Florida Keys versus samples from the Gulf; tests of homogeneity among all samples, among samples from the Gulf, and between pooled samples from the Atlantic versus those from the Gulf plus the Florida Keys were nonsignificant (Table 4). Estimates of $F_{ST}$ revealed the same pattern; the $F_{ST}$

**Table 5**

Hierarchical analysis of molecular variation (AMOVA) among mtDNA haplotypes of greater amberjack (*Seriola dumerili*) from the Gulf of Mexico and U.S. southeastern Atlantic.

| Variance component | Observed partition | | $P^1$ | $\Phi$ values |
|---|---|---|---|---|
| | Variance | % total | | |
| Gulf + Florida Keys vs. Atlantic | | | | |
| Between regions | 0.00127 | 0.29 | 0.259 | $\Phi_{CT} = 0.003$ |
| Among samples within regions | 0.00160 | 0.36 | 0.190 | $\Phi_{SC} = 0.004$ |
| Within samples | 0.43974 | 99.35 | 0.121 | $\Phi_{ST} = 0.006$ |
| Atlantic + Florida Keys vs. Gulf | | | | |
| Between regions | 0.00398 | 0.90 | 0.001 | $\Phi_{CT} = 0.009$ |
| Among samples within regions | 0.00011 | 0.03 | 0.444 | $\Phi_{SC} = 0.000$ |
| Within samples | 0.43974 | 99.07 | 0.113 | $\Phi_{ST} = 0.009$ |

[1] Probability of finding a more extreme variance component by chance alone (5000 permutations).

value of 0.009 in the pooled comparison of samples from the Atlantic and Florida Keys versus samples from the Gulf differed significantly from zero, whereas $F_{ST}$ values in all other comparisons were nonsignificant (Table 4).

For AMOVA, where the variance in mtDNA haplotypes was partitioned by region, we also performed two separate analyses: one where the sample from the Florida Keys was included with Gulf samples, and one where the sample from the Florida Keys was included with Atlantic samples. In both analyses, the majority of the variance (>99%) was distributed within samples, and in both, the among-samples-within-groups component ($\Phi_{SC}$) was nonsignificant (Table 5). The between-region component ($\Phi_{CT}$) in the comparison of Atlantic and Florida Keys versus the Gulf was highly significant ($P<0.001$); whereas $\Phi_{CT}$ in the comparison of Atlantic versus the Gulf plus Florida Keys was not (Table 5). These results paralleled results of the homogeneity tests and the $F_{ST}$ values. Finally, we employed pairwise $\Phi_{ST}$ values (i.e. between pairs of samples) to generate average "distance" values between samples from the Gulf (eight total), between samples from the Atlantic plus the sample from the Florida Keys (three total), and between samples from the Gulf versus samples from the Atlantic (and the Florida Keys). The average (±SE) $\Phi_{ST}$ values for these comparisons were 0.004 ±0.001 (samples from the Gulf), 0.002 ±0.002 (samples from the Atlantic and the Florida Keys), and 0.012 ±0.002 (samples from the Gulf versus samples from the Atlantic and the Florida Keys).

Homogeneity ($V$) tests were carried out on the ten haplotypes (1–4, 6, 11, 13, 23, 37, and 48) that were found in at least six individuals. Over all 11 samples, only haplotype 6 differed significantly ($P$~0.048) in frequency. This result is not significant when corrections for multiple testing (Rice, 1989) are applied. We then pooled samples into two groups (one that included the eight samples from the Gulf, and one that included the two samples from the Atlantic and the sample from the Florida Keys). Significant $V$ tests (again, prior to corrections for multiple testing) were found for haplotype 6 ($P$~0.011) and haplotype 13 ($P$~0.024). Frequency plots by sample of these two haplotypes (Fig. 2) revealed a clinal pattern to variation in haplotype 6, with frequencies generally increasing eastwardly in the Gulf and into the Atlantic; no pattern was apparent for variation in haplotype 13.

Maximum-parsimony (MP) analysis of individual mtDNA haplotypes and neighbor-joining (NJ) analysis of the matrix of interpopulational (genetic) distances revealed no evidence of phylogeographic structure. MP analysis generated an unresolved multichotomous topology where a few clades of individual mtDNA haplotypes were supported at 90% or greater in bootstrap analysis (100 replicates). In each case, haplotypes within such clades were not geographically cohesive. Examples included a clade of haplotypes 5, 52, and 47, which were found, respectively, in waters off Texas and west Florida (5), South Carolina (52), and the Florida Keys (47) (Table 2); a clade of haplotypes 32, 39, and 46, which were found, respectively, in waters off Texas and Louisiana (42), east Florida (39), and Texas and west Florida (46) (Table 2); and a clade of haplotypes 22, 27, 37, and 51, which were found, respectively, in waters off west Florida (22), Texas (27), several localities (37), and west Florida and South Carolina (51) (Table 2). No "deep" subdivisions, in the sense of multiple restriction sites identifying a clade of haplotypes, were evident. The NJ topology (Fig. 3) indicated relatively

long terminal branches to individual samples, but short internodal branches linking samples. Samples did not necessarily cluster geographically, and in no case did geographically proximate samples form a cluster.

Spatial autocorrelation analyses generated 40 Moran's $I$ values in each run (10 haplotypes × four distance classes). Four significant ($P<0.05$) values were obtained when equal numbers of pairwise comparisons were used: two were positive and occurred in the first and third distance classes, and two were negative and occurred in the third and fourth distance classes. Two significant values ($P<0.05$) were obtained when equal geographic distances were used: one in the first distance class was positive, and one in the third distance class was negative. Mean $I$ values were negative in all four distance classes in both runs and did not differ significantly from expected values of $I$ in the absence of autocorrelation (Fig. 4).

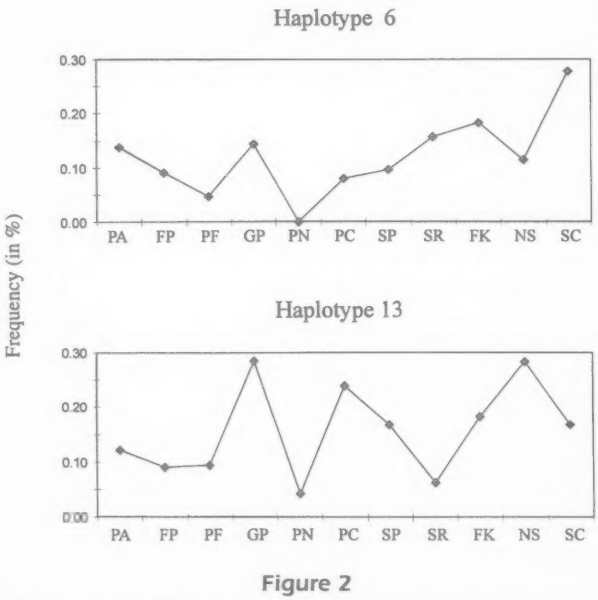


**Figure 2**

Frequency of mtDNA haplotypes 6 and 13 among greater amberjack (*Seriola dumerili*) sampled from 11 localities in the northern Gulf of Mexico and southeastern (U.S.) Atlantic. Acronyms are defined in Table 1.

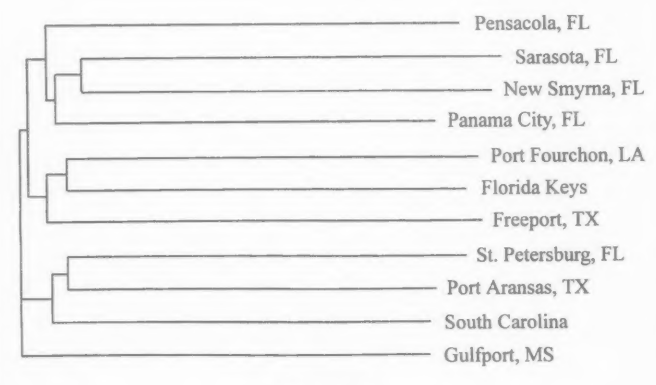


**Figure 3**

Neighbor-joining topology generated from matrix of pairwise, interpopulational (mtDNA) nucleotide sequence divergence among samples of greater amberjack, *Seriola dumerili*.

## Discussion

Variation of mtDNA in greater amberjack is consistent with the hypothesis that one subpopulation (stock) exists in the northern Gulf of Mexico (Gulf), whereas a second subpopulation (stock) exists along the U.S. southeast Atlantic coast (Atlantic). The latter (Atlantic) subpopulation includes the sample from the Florida Keys. Evidence supporting this hypothesis includes 1) significant heterogeneity in the distribution of pooled haplotypes (and of haplotype 6) from the Gulf versus those from the Atlantic (and the Florida Keys); 2) a significant (nonzero) $F_{ST}$ value in the same comparison; 3) a highly significant ($P<0.001$) between-region component ($\Phi_{CT}$ value) in AMOVA, when regions were defined as the Gulf versus the Atlantic (and the Florida Keys); and 4) comparison of pairwise $\Phi_{ST}$ "distances" where samples from the Gulf or from the Atlantic were at least three times more similar to one another than were samples from the Gulf versus those from the Atlantic (and the Florida Keys).

Neither maximum-parsimony (MP) analysis of individual mtDNA haplotypes nor neighbor-joining analysis of a matrix of interpopulational (intersample) nucleotide-sequence distances revealed phylogeographic patterns indicative of population structuring. A few, well-supported clades of individual haplotypes were detected in MP analysis, but in no case were haplotypes within individual clades from the same or geographically proximate

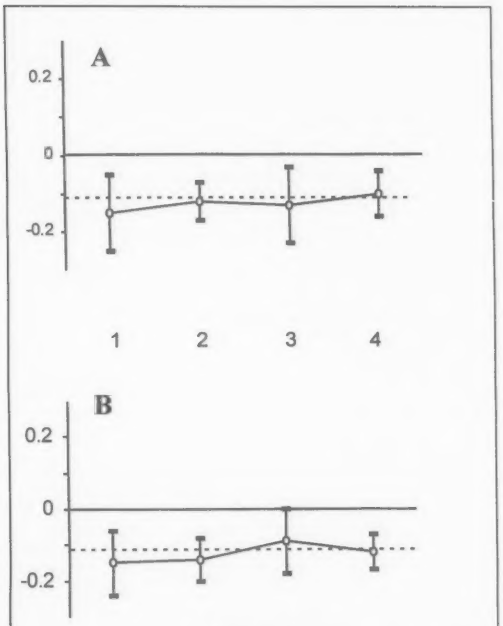


**Figure 4**

Correlograms based on frequencies of mtDNA haplotypes found in eight or more individuals among samples of greater amberjack, *Seriola dumerili*, from the northern Gulf of Mexico. Abscissas: distance classes 1–4 (left to right); ordinates: mean autocorrelation coefficients (Moran's *I* values) for each distance class (±SE). (**A**) Equal frequencies/distance class; (**B**) equal distances between distance classes.

sample localities. Absence of structure in MP analysis is not inconsistent with results of homogeneity testing, $F_{ST}$ values, and AMOVA analysis, in that 1) presumed restrictions in gene flow between subpopulations could be relatively recent, i.e. there has been insufficient time for haplotypes (e.g. haplotype 6) that differ in frequency between subpopulations to become reciprocally monophyletic, or 2) there may be limited gene flow between subpopulations. Finally, there was no indication of spatial autocorrelation (positive or negative) in common haplotypes among samples from the Gulf. This finding indicates the absence of an isolation-by-distance effect among greater amberjack in the Gulf and is consistent with the hypothesis of continuous gene flow across the northern Gulf.

Divergence in mtDNA between Gulf and Atlantic subpopulations has been documented for a variety of marine species. In some (e.g. American oysters, toadfish, black sea bass, and to a lesser extent, horseshoe crabs), major phylogeographic discontinuities between Gulf and Atlantic subpopulations were found, leading to the hypothesis that the similar vicariant patterns may have stemmed from episodic changes in environmental conditions during Pleistocene glaciation (Avise, 1992). In addition, the presence of phylogeographic structure was taken to indicate that current-day gene flow between subpopulations is very restricted, if it occurs at all. In species such as red drum (Gold et al., 1993), king mackerel (Gold et al., 1997), and greater amberjack (our study), mtDNA differences between Gulf and Atlantic subpopulations are documented but are limited to either a frequency difference in a single mtDNA haplotype, a small (but significant) difference in mtDNA haplotype distribution, or both. The relatively small genetic differences observed between subpopulations in these species, along with the absence of phylogeographic structure, both between regions and among haplotypes, suggest either that limited gene flow occurs between subpopulations or that separation between subpopulations is fairly recent. In the case of greater amberjack, the former is consistent with mark-and-recapture data that suggest exchange rates of 1.5% between Gulf and Atlantic stocks (Cummings and McClellan[3]).

For greater amberjack, the boundary between Gulf and Atlantic subpopulations appears to be between the Florida Keys (included in the Atlantic subpopulation) and somewhere off the central-western Florida coast, possibly the Florida Middle Ground, a series of north-south reef structures located about 150 km south of the north Florida coast and about 160 km northwest of Tampa Bay (Hopkins et al.[6]), and the primary source for amberjack fishermen located in Sarasota and St. Petersburg. Separation between Gulf and Atlantic subpopulations (stocks) of greater amberjack could stem from a number of causes that involve historical or recent interactions between dispersal capability and impediments to gene flow, or both. Among present-day alternatives are 1) offshore currents that are not conducive to unrestricted movement between regions; and 2) absence of suitable habitat or difference in ecological (biogeographic) provinces between regions. A third (historical) possibility might be that subpopulations were separated (e.g. during Pleistocene glaciations) and have only recently (in geological time) begun exchanging genes. Rates of approach to genetic homogeneity under this last hypothesis are, in part, time-dependent, and one could speculate that there has been insufficient time for accumulated genetic differences to disappear.

[6] Hopkins, T. 1981. Florida Middle Ground. *In* R. Rezak and T. J. Bright (eds.), Final report: northern Gulf of Mexico topographic features study, p. 1–5. Tech. Rep. No. 81-2-T, Dep. Oceanography, Texas A&M University, College Station, TX.

There is at least suggestive evidence for each of the above three possibilities in greater amberjack. First, measurements of offshore current velocities are consistent with increased passive movement of individuals from the Florida Keys into the Atlantic rather than northward along the west Florida coast. Mean current velocities (145 m below the surface) eastward from the Keys through the Florida Straits are approximately 75 cm/sec, and along the southeast Florida coast, these currents can be as fast as 95 cm/sec (SAIC[7]). Because current velocities are expected to be greater nearer the surface, and because greater amberjack have been observed spawning in the Florida Keys (Cummings and McClellan[3]), buoyant eggs and larvae would likely be impacted and have little opportunity to move northward along the west coast of Florida. Second, in terms of habitat, the southern half of the Florida peninsula represents a transition zone between temperate and tropical forms, where the southern ranges of many temperate species terminate in tropical southern Florida (Briggs, 1974). There also is relatively little reef habitat or sharp topography between the Florida Middle Ground and the Keys; most of the area has a relatively smooth bottom comprising shell, sand, and quartz (Rezak et al., 1985; Rezak and Bright[8]). Because greater amberjack exhibit a marked preference for reefs, rock outcrops, and wrecks (Shipp, 1986; Manooch, 1988), it is possible that the paucity of significant reef or other major structure on the Outer Continental Shelf off southwestern Florida may inhibit movement of greater amberjack between the Florida Middle Ground and the Keys. Finally, although relatively little is known about the early life history of greater amberjack, spawning is thought to occur from mid-spring to early summer both along the southeastern coast of Florida (including the Florida Keys) and in the northern Gulf off Louisiana (Cummings and McClellan[3]; Thompson[9]). This spawning time suggests that warmer water temperatures may trigger onset of reproductive activity. One might then hypothesize that during the late Pleistocene Epoch, when waters of the northern Gulf were much cooler (Rezak et al., 1985), subpopulations of greater amberjack were isolated in warm-water refugia, perhaps off the Florida Keys (or in the Caribbean) and off the Yucatan Peninsula (Campeche Banks) where considerable reef habitat exists (Rezak et al., 1985). Following glacial retreat, the (putatively) isolated subpopulations could then have returned to the northern Gulf. Because the rate of approach to genetic homogeneity would be partly a function of time and gene flow, genetic differences between present-day subpopulations of greater amberjack could simply be historical artifacts, reflecting insufficient time (or restricted gene flow) relative to genetic homogenization.

Our finding that the sample of greater amberjack from the Florida Keys was included in a grouping (subpopulation) with samples from the Atlantic differs from a recent study in king mackerel (Gold et al., 1997), where a sample from the Florida Keys was placed in a grouping with samples from the Gulf. King mackerel are highly migratory, and the sample of king mackerel from the Florida Keys was taken during late winter when the majority of king mackerel in the Keys are thought to be from the Gulf Migratory Unit or stock (Williams and Godcharles[10]). The sample of greater amberjack from the Keys, however, was obtained during late March–early April, a time when the majority of king mackerel in the Keys are considered to be from the Atlantic Migratory Unit or stock (Williams and Godcharles[10]). Although greater amberjack are not migratory in the same way as king mackerel, it would be of more than passing interest to examine winter samples of greater amberjack from the Florida Keys and ask whether the Florida Keys constitute a mixing zone in greater amberjack as in king mackerel. Along these lines, it also would be important to examine both summer and winter samples of greater amberjack in the area between the Florida Middle Ground and the Florida Keys. A better definition of the geographic limits of the two subpopulations is critical for assessment and allocation of the greater amberjack resources along the west coast of Florida.

Avise et al. (1988) presented models that allow estimation of evolutionary (long-term) effective female-population size ($N_{f(e)}$ values) based on mtDNA intrapopulational nucleotide sequence diversities. Assuming that the generation time in greater amberjack is three years (Wilson[11]), we estimated $N_{f(e)}$ for the two subpopulations (stocks) of greater amberjack to be 90,000 (Gulf of Mexico) and 93,500 (U.S. southeastern Atlantic, including the Florida Keys).

[7] SAIC (Science Applications International Corporation). 1992. Straits of Florida physical oceanographic field study, final interpretative report, volume II: technical report. OCS Report/MMS 92-0024. U. S. Dep. Interior, Minerals Mgmt. Serv., Gulf of Mexico OCS Regional Office, New Orleans, LA, 179 p.

[8] Rezak, R., and T. J. Bright (eds.). 1981. Final report: northern Gulf of Mexico topographic features study. Tech. Rep. No. 81-2-T, Dept. Oceanography, Texas A&M University, College Station, TX, 150 p.

[9] Thompson, B. A. 1997. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803. Personal commun.

[10] Williams, R. O., and M. F. Godcharles. 1984. Completion report, king mackerel tagging and stock assessment. Project 2-341-R. Florida Dep. Natural Resources., St. Petersburg, FL.

[11] Wilson, C. A. 1997. Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803. Personal commun.

The two estimates do not differ significantly from one another and are larger than those reported in a more limited (in terms of sample size and geographic coverage) study of greater amberjack (Richardson and Gold, 1993). They also are commensurate with $N_{f(e)}$ estimates for several other marine fish of commercial or recreational value (Gold et al., 1993). Although a positive correlation exists between effective population sizes and census sizes, the latter are generally an order of magnitude or two larger than the former (Avise et al., 1988). One reason for this difference, at least in estimates of $N_{f(e)}$ values for poikilothermic vertebrates, is that the models of Avise et al. (1988) employ (estimated) mtDNA evolutionary rates for homeothermic vertebrates. Estimated mtDNA evolutionary rates for poikilothermic vertebrates may be 5–10 times less than those for homeothermic vertebrates (Martin and Palumbi, 1993), suggesting that long-term effective population sizes of each subpopulation of greater amberjack could be on the order of 500,000 to 1,000,000 females.

According to results of this project, current stock boundaries for assessment and allocation of greater amberjack resources in U.S. waters appear appropriate except possibly for the west coast of Florida, south of the Florida Middle Ground. Depending on present or future importance of the greater amberjack fishery in this area, it would be useful to know both the geographic limits of the two subpopulations and whether the Florida Keys constitute a mixing zone for greater amberjack as for species such as king mackerel.

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## Literature cited

**Avise, J. C.**
**1992**. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

**Avise, J. C., R. M. Ball, and J. Arnold.**
**1988**. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. 5:331–344.

**Berry, F. H., and R. K. Burch.**
**1978**. Aspects of the amberjack fisheries. Proc. Gulf Caribb. Fish. Inst. 31:179–194.

**Bohlmeyer, D. A., and J. R. Gold.**
**1991**. Genetic studies in marine fishes. II. A protein electrophoretic analysis of population structure in the red drum *Sciaenops ocellatus*. Mar. Biol. 108:197–206.

**Briggs, J. C.**
**1974**. Marine zoogeography. McGraw Hill, New York, NY, 475 p.

**Camper, J. D., R. C. Barber, L. R. Richardson, and J. R. Gold.**
**1993**. Mitochondrial DNA variation among red snapper (*Lutjanus campechanus*) from the Gulf of Mexico. Mol. Mar. Biol. Biotechnol. 3:154–161.

**DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston.**
**1987**. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. Genetics 116:215–223.

**Excoffier, L., P. E. Smouse, and J. M. Quattro.**
**1992**. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

**Felsenstein, J.**
**1989**. PHYLIP (Phylogeny Inference Package), version 3.4. Cladistics 5:164–166.

**Gold, J. R., and L. R. Richardson.**
**1991**. Genetic studies in marine fishes. IV. An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. Fish. Res. 12:213–241.

**Gold, J. R., Á. Ý. Kristmundsdóttir, and L. R. Richardson.**
**1997**. Mitochondrial DNA variation in king mackerel (*Scomberomorus cavalla*) from the western Atlantic Ocean and Gulf of Mexico. Mar. Biol. 129:221–232.

**Gold, J. R., L. R. Richardson, C. Furman, and T. L. King.**
**1993**. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. Mar. Biol. 116:175–185.

**GMFMC (Gulf of Mexico Fishery Management Council).**
**1989**. Amendment number 1 to the reef fish fishery management plan. Gulf of Mexico Fishery Management Council, Tampa, FL, 356 p.

**Hilborn, R.**
**1985**. Apparent stock-recruitment relationships in mixed stock fisheries. Can. J. Fish. Aquat. Sci. 42:718–723.

**Manooch, C. S., III.**
**1988**. Fisherman's guide: fishes of the southeastern United States. NC State Mus. Nat. Hist., Raleigh, NC, 362 p.

**Martin, A. P., and S. R. Palumbi.**
**1993**. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. (USA) 90:4087–4091.

**McElroy, D., P. Moran, E. Bermingham, and I. Kornfield.**
**1992**. REAP-The restriction enzyme analysis package. J. Hered. 83:157–158.

**Nei, M., and J. C. Miller.**
**1990**. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction site data. Genetics 125:873–879.

**Nei, M., and F. Tajima.**
**1981**. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

**Rezak, R., T. J. Bright, and D. W. McGrail.**
**1985**. Reefs and banks of the northwestern Gulf of Mexico. John Wiley & Sons, New York, NY, 259 p.

**Rice, W. R.**
**1989**. Analyzing tables of statistical tests. Evolution 43: 223–225.

**Richardson, L. R., and J. R. Gold.**
**1993**. Mitochondrial DNA variation in red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*) from the Gulf of Mexico. ICES J. Mar. Sci. 50:53–62.

**Roff, D. A., and P. Bentzen.**
**1989**. The statistical analysis of mitochondrial polymorphisms: chi-square and the problem of small samples. Mol. Biol. Evol. 6:539–545.

**Saitou, N., and M. Nei.**
**1987**. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

**Shipp, R. L.**
**1986**. Dr. Bob Shipp's guide to the fishes of the Gulf of Mexico. 20th Century Printing Co., Mobile, AL, 256 p.

**Sinclair, M., V. C. Anthony, T. D. Iles, and R. N. O'Boyle.**
**1985**. Stock assessment problems in Atlantic herring (*Clupea harengus*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 42:888–898.

**Smith, P. J., and Y. Fujio.**
**1980**. Genetic variation in marine teleosts: high variability in habitat specialists and low variability in habitat generalists. Mar. Biol. 69:7–20.

**Sokal, R. R., and F. J. Rohlf.**
**1969**. Biometry: the principles and practice of statistics in biological research. Freeman and Sons, San Francisco, CA, 776 p.

**Southern, E. M.**
**1975**. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

**Swofford, D. L.**
**1991**. PAUP: Phylogenetic analysis using parsimony. Users manual. Illinois Nat. Hist. Surv., Champaign, IL, 179 p.

**Waples, R. S.**
**1987**. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400.

**Wartenberg, D.**
**1989**. SAAP: a spatial autocorrelation analysis program. Dep. Environ. Community Med., Robert Wood Johnson Medical School, Univ. of Medicine and Dentistry of New Jersey, Piscataway, NJ, 28 p.

**Weir, B. S.**
**1990**. Genetic data analysis. Sinauer Assoc., Inc., Sunderland, MA, 377 p.

**Weir, B. S., and C. C. Cockerham.**
**1984**. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

# Age, growth, and reproduction of the tropical squid *Nototodarus hawaiiensis* (Cephalopoda: Ommastrephidae) off the North West Slope of Australia

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**Abstract.**—The age, growth, and reproduction of the tropical Indo-Pacific ommastrephid squid *Nototodarus hawaiiensis* was studied on the North West Slope of Australia. The weight, mantle length (ML), gonad weight, and nidamental gland length were measured for 37 males and 52 females captured in January and February 1992 and ranging in size from 42 mm ML to 214 mm ML. Statolith increments were counted, as a proxy for age. The number of statolith increments, counted on 42 of the squid, ranged from 49 to 195. The relation between increment number (i.e. age) and ML was linear for both sexes. The relation between increment number and ovary weight, and between increment number and testis weight, had greater variability than did ML versus ovary weight, and ML versus testis weight, indicating a large range in age at maturity in individuals of similar size. Some statoliths showed two prominent zones, the origins of which are uncertain. Back-calculated hatch dates indicated that all squid hatched between July and December 1991 and that the majority hatched between August and October (Austral spring).

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Fish. Bull. 96:779–787 (1998).

*Nototodarus hawaiiensis* (Berry, 1912) was thought originally to be restricted to the Hawaiian Islands in the central Pacific (Roper et al., 1984). However, studies by Dunning (1988a, 1988b) revealed that this species is also distributed in the Indo-Pacific including the Philippines (previously referred to as *N. sloani philippinensis* [Voss, 1962]), northern Australia, South China Sea, western Indian Ocean, and off Chile (Dunning and Förch, in press). In Australian waters, postparalarval *N. hawaiiensis* inhabit slope waters at depths between 200 and 500 m off the North West Slope and between 100 and 600 m off the north east coast to southern Queensland (Dunning, 1988b) in bottom temperatures of 12.4°C. Recent trawl surveys on the North West Slope of Australia have shown that *N. hawaiiensis* is the dominant species of cephalopod in commercial catches from demersal trawlers (Wadley, 1993). The recent identification and dominance of *N. hawaiiensis* in waters off the North West Slope of Australia necessitates understanding the biology and role of this species in deepwater marine communities in tropical Australian waters.

This study was carried out to obtain preliminary age, growth, and reproductive parameters for *N. hawaiiensis* not previously reported. Statolith age analysis, a valuable tool in squid growth and life history studies (Jackson, 1994), was carried out as an indication of age and growth. Previous statolith ageing work on tropical Australian squid species has been restricted to shallow water loliginids that predominantly complete their life span in less than 200 d (Jackson, 1990; Jackson and Choat, 1992; Jackson and Yeatman, 1996). However, no growth information has been available to date for deepwater tropical species of other families. It was therefore of interest, for comparative purposes, to obtain life history parameters of this deepwater tropical ommastrephid.

Statoliths of *N. hawaiiensis* were studied to assess if periodic increments might be useful for ageing this species. Owing to the difficulty of obtaining live specimens from deep water, age information has to be inferred (as in this study), rather than validated on living squid. Increment structure was considered in relation to the validation evidence available for other ommastrephids.

## Material and methods

### Squid collection and analysis

Individuals were collected day and night, 20 January to 14 February 1992, off the North West Slope of Australia, between about 12°S and 22°S (Wadley, 1993) and 385 to 555 m. This study was composed of 89 individuals of which 42 individuals were used for age analysis. Specimens were obtained from the RV *Southern Surveyor* and commercial vessels, which trawled with demersal gear (45-mm mesh net) on soft sandy substrates.

The fresh *N. hawaiiensis* were identified by using field characters from Wadley (1990). Other ommastrephids were captured (Wadley, 1993) but *Nototodarus gouldi* was absent in the area. Specimens were sampled at random from the range of sizes available in the trawl catches. The squid were frozen at sea and subsequently defrosted in the laboratory for reproductive and statolith analysis. Measurements taken included mantle length (ML), total wet weight (W), testis weight for males and ovary weight and nidamental gland length (NGL) for females. Hectocotylisation of the ventral arm and presence of spermatophores in Needham's sac were used as indicators of sexual maturity in males. In females, development of the nidamental gland and presence of mature oocytes in the ovaries were used as indicators of sexual maturity.

### Statolith removal and analysis

Statoliths were removed through an incision in the cephalic cartilage, placed singly on microscope slides, and rinsed with 100% ethanol. The dried statoliths were mounted in thermoplastic cement (Crystal Bond, Jackson, 1990). Statoliths were taken for age analysis from 42 individuals selected from the full size range available.

To observe the increment structure, statoliths were ground and polished, usually on both the anterior and posterior surfaces. The complete increment sequence was usually visible only on the posterior (convex) surface, which was preferred for routine counting. In many of the statoliths, the crystal structure obstructed a view of the increments in the nuclear region of the anterior surface. Some statoliths were ground from the anterior (concave) surface through the obstructing crystals until the nucleus was revealed and all the increments could be observed (terminology according to Lipinski et al., 1991).

Statoliths were viewed on a computer monitor with a video camera attached to a compound microscope. Increments were counted by following the increment sequence with a cursor while using a hand counter. The number of increments on each statolith was counted at least three times and the mean was taken. Counts that varied more than 10% from the mean were repeated or rejected. The increments were similar in structure to daily statolith increments observed in other ommastrephid species (e.g. Hurley et al., 1985; Villanueva, 1992; Arkhipkin, 1996).

## Results

### Length-weight relationship

Weight and length parameters were measured for 37 males and 52 females. Males ranged in size from 42 mm ML and 6.6 g to 176 mm and 211.7 g; females ranged from 48 mm ML and 5.3 g to 214 mm ML and 381.6 g (Fig. 1).

### Increment number

The relation between the number of statolith increments and ML was linear for both males and females over the size range available, although there was considerable variability in the data (Fig. 2). The regression equations were

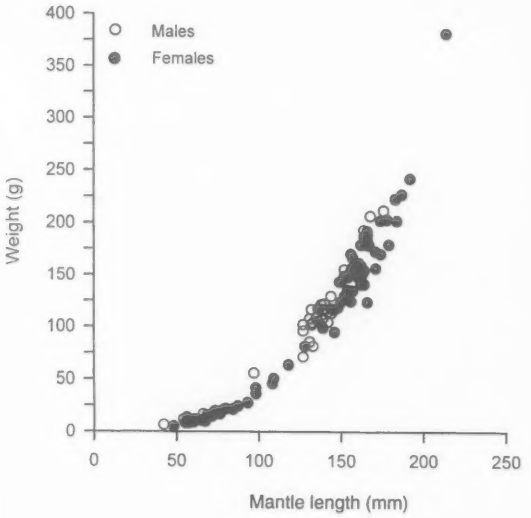


**Figure 1**

The relation between mantle length and weight for males and females of *Nototodarus hawaiiensis*.

$$y = 0.95x - 7.75 \qquad (r^2 = 0.75)$$

and

$$y = 1.08x - 14.915 \qquad (r^2 = 0.78),$$

for males and females respectively,
where $x$ = increment number; and
$y$ = mantle length (mm).

The increment count for males ranged from 71 (42 mm ML) to 192 (164 mm ML). Statolith increments in the largest male collected (176 mm ML) could not be counted owing to overgrinding of the statolith. The increment count for females ranged from 49 (48 mm ML) to 195 (183 mm ML). The largest female (214 mm ML) had an increment count of 179.

## Male maturation patterns

The largest immature male was 127 mm ML and had 167 statolith increments. Males appeared to mature as early as 100 d and 90 mm ML. Mature males showed considerable range in weights of testis (from 0.92 g to 2.84 g, Fig. 3A).

On the basis of ML alone, testis weight appeared to increase rapidly with growth (Fig. 3A), and there was some evidence of testis regression (on the basis of reduced testis weight relative to ML) at larger sizes. However, when testis growth was compared with increment number, there was no clear pattern. Plotting testis weight against increment number showed that mature individuals varied widely in age and individuals of similar age varied widely in testis weight (Fig. 3B).

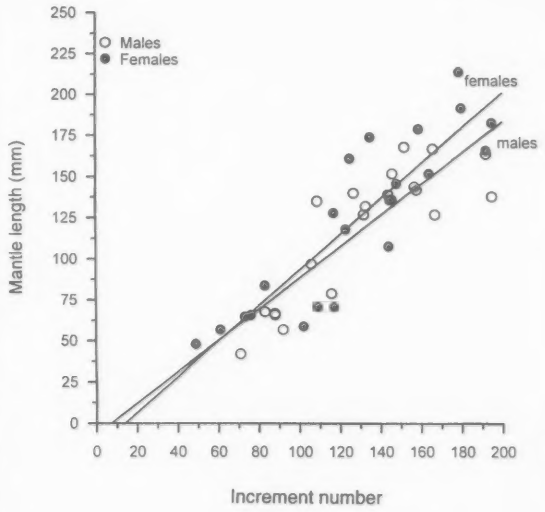


**Figure 2**

The relation between statolith increment number and mantle length for males and females of *Nototodarus hawaiiensis*. Regression equations are given in the text.

## Female maturation patterns

The largest immature female was 166 mm ML and had 192 statolith increments, whereas the smallest mature female was 136 mm ML and had 146 statolith increments. All females larger than 166 mm ML were mature on the basis of the presence of mature oocytes. On the basis of ML (Fig. 4A), females reached full maturity over a narrow length range between approximately 136 and 170 mm ML. However, as with testis weight for the males, ovary weight plotted against increment number (Fig. 4B) showed considerably more variability than did ovary weight plotted against ML (Fig. 4A). The youngest mature female was 125 d. It appeared that there was little growth in the ovary before 100 d.

The largest immature female (166 mm ML) had a NGL of 48 mm ML. Whereas the smallest mature female (136 mm ML) had a NGL of 51 mm, all mature females had a NGL greater than 38 mm. All immature individuals had a NGL less than 50 mm.

The trend in growth of the nidamental gland followed a similar pattern for either ML or increment number (Fig. 5, A and B).

## Hatching dates

On the assumption that statolith increments are formed daily, hatching dates were backcalculated. All individuals that were aged hatched between July and December 1991; the majority (83%) hatched between August and November, and 71% hatched in August, September, and October (Fig. 6). Most squid hatched during the austral spring (September–November), although there was some hatching in late winter (July) and early summer (December).

## Statolith microstructure and growth zones

The statoliths of *N. hawaiiensis* generally had clear increments that could be counted from the nucleus to the outer margin of the dorsal dome (Fig. 7). There was a pattern in the zonation in nine of the 42 statoliths examined (Fig. 7, A and B). Some individuals had a distinct inner opaque zone followed by an outer translucent zone (Fig.

7). No obvious opacity was observed in any of the juvenile (<110 mm ML, $n$=14) statoliths. Opaque zone increment counts on the nine individuals ranged between 89 and 135.

The crystal structure obscuring increments in some statoliths of *N. hawaiiensis* resembled crystals in other squid statoliths. They appeared similar to structures in *Illex illecebrosus*, described as "nodules" by Lipinski (1981) or "occulting crystals" by Dawe et al. (1985). Similar crystals were also observed in the statolith microstructure of the Antarctic squid *Mastigoteuthis psychrophila* (Jackson and Lu, 1994).

## Discussion

### Maturation

*Nototodarus hawaiiensis* matures at a relatively young age (<150 d). According to our study of trawl-caught specimens, *N. hawaiiensis* may have a life span of less than 200 d. This is in contrast to its temperate-water conspecifics *Nototodarus gouldi* and *N. sloanii*, which do not reach maturity until 200 d in New Zealand waters (Uozumi et al., 1995). Maturation in *N. hawaiiensis* appears to be more closely tied

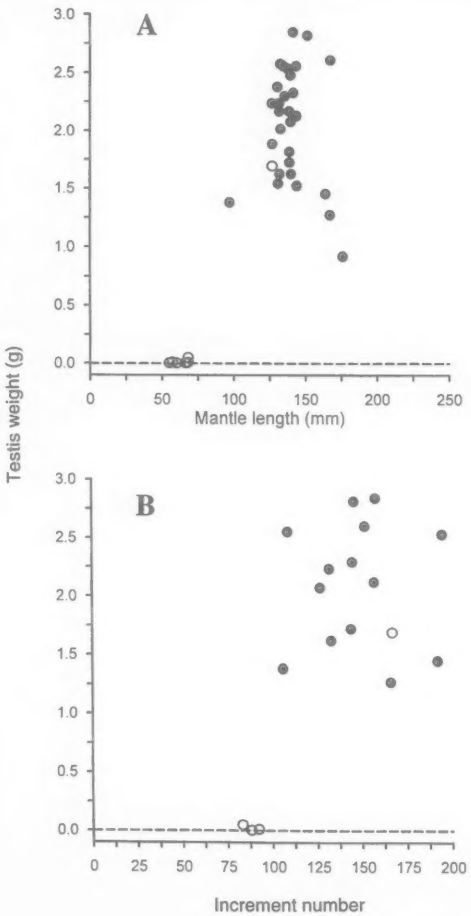


**Figure 3**

The relation between (**A**) mantle length and testis weight, and (**B**) increment number and testis weight for *Nototodarus hawaiiensis*. Filled circles represent mature individuals; hollow circles represent immature individuals.

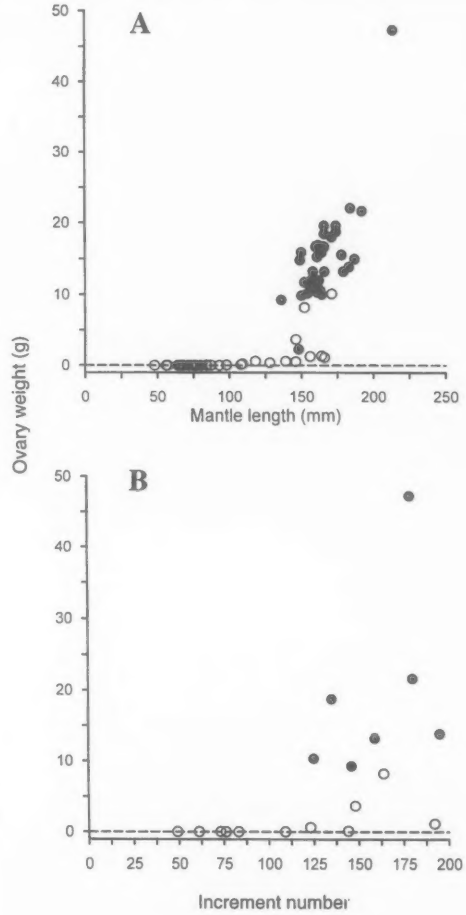


**Figure 4**

The relation between (**A**) mantle length and ovary weight, and (**B**) increment number and ovary weight for *Nototodarus hawaiiensis*. Filled circles represent mature individuals; hollow circles represent immature individuals.

to body size than to age, suggesting that there is a minimal physical or physiological size threshold to be reached before maturity can take place, regardless of age. This pattern has also been found in shallow-water *Photololigo* sp. (referred to as *Loligo chinensis* in Jackson, 1993a) and *Lolliguncula brevis* (Jackson et al., 1997), as well as in males of the deepwater onychoteuthid *Moroteuthis ingens* (Jackson, 1997). Guerra and Castro (1994) found that female reproductive organs of *Loligo gahi* generally required a minimum body size before increasing substantially in size. Such a trend may be a common strategy in squids. *Illex argentinus* in the South Atlantic (Rodhouse and Hatfield, 1990) likewise shows considerable variability in the timing of maturation in relation to age. Rodhouse and Hatfield (1990) postulated that for *I. argentinus*, maturity and gonad growth does not occur at the expense of somatic growth. This also appears to be the case for *N. hawaiiensis*. However, this pattern of maturation contrasts with the deepwater onychoteuthid squid *Moroteuthis ingens*, which undergoes degradation of somatic tissues with maturation (Jackson and Mladenov, 1994).

Size of the nidamental gland relative to body length in *N. hawaiiensis* appears to be a useful indication of female maturity because growth of this organ is closely associated with growth of the ovary (Ikeda et al., 1991, Collins et al., 1995). Uozumi et al. (1995) found a close association in growth of the nidamental gland and ovary size and ovulation in the closely related *Nototodarus sloanii* and *N. gouldi* in New Zealand.

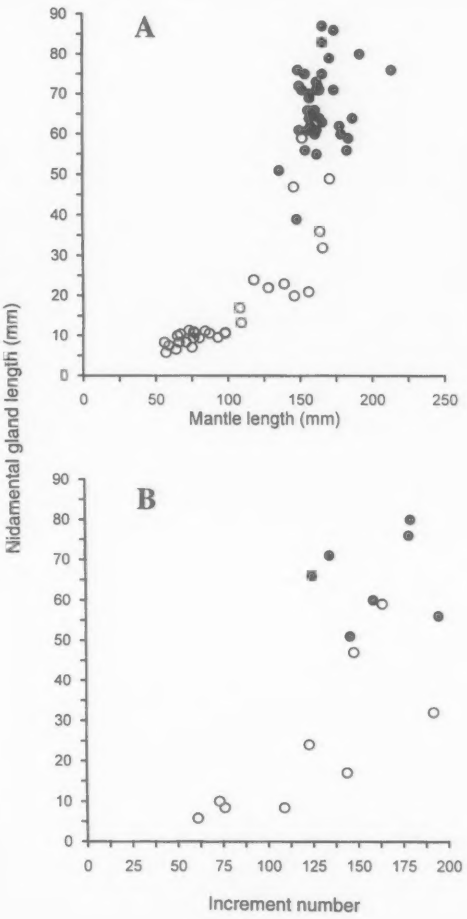


**Figure 5**

The relation between (**A**) mantle length and nidamental gland length and (**B**) increment number and nidamental gland length for *Nototodarus hawaiiensis*. Filled circles represent mature individuals; hollow circles represent immature individuals.

## Hatching

Ageing data suggest that some ommastrephids have extended spawning periods (e.g. *Illex argentinus*, Arkhipkin, 1993; *Todarodes pacificus*, Nakamura and Sakurai, 1993; *Todarodes sagittatus, Nototodarus sloanii,* Uozumi and Ohara, 1993; *Ommastrephes bartramii*, Bower, 1996). Other ommastrephids, such as *Illex illecebrosus,* which hatches predominantly in spring (Dawe and Beck, 1997), appear to have peaks of spawning. In some instances, spawning peaks may be regionally influenced; *Martialia hyadesi* captured on the Patagonian Shelf Edge had

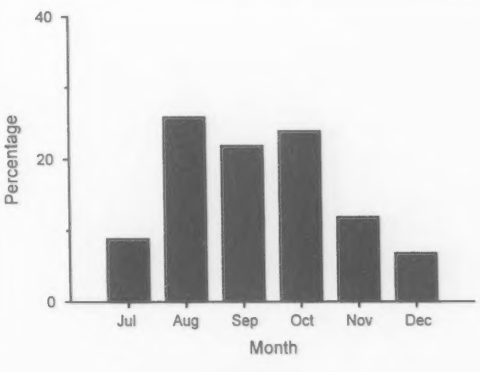


**Figure 6**

The hatching-date distribution of all individuals ($n$=42) of *Nototodarus hawaiiensis* aged in this study.

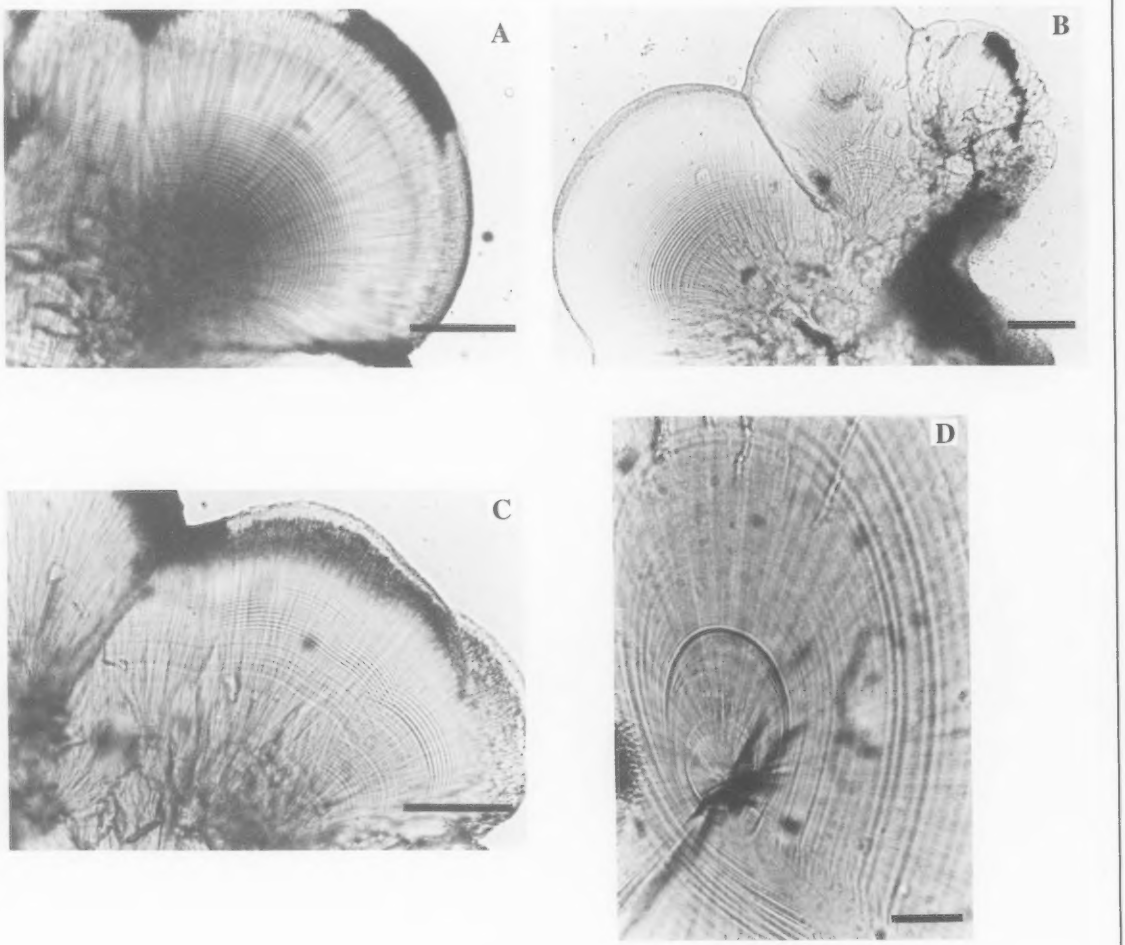


**Figure 7**

Statolith microstructure of *Nototodarus hawaiiensis* (**A**) dorsal dome region of statolith male (132 increments, 127 µm ML)—note the distinct inner opaque zone followed by the outer translucent zone, scale bar = 100 µm (**B**) dorsal dome region of statolith of female (166 µm ML, increment count not taken) —note the less distinct opaque and translucent zones, scale bar = 100 µm (**C**) dorsal dome region of statolith of female (146 increments, 136 µm ML)— note lack of any obvious opaque zone and the unusual overlapping increment structure shown to the right of the photograph, scale bar = 100 µm (**D**) nuclear region of statolith of juvenile female (61 increments, 57 µm ML), scale bar = 25 µm.

a spring peak, whereas individuals captured at the Antarctic Polar Front hatched in winter (Rodhouse et al., 1994). This preliminary data indicates a late winter–spring to early summer hatching for *N. hawaiiensis* in this study. Wadley (1993) collected 50-mm-ML juveniles of *N. hawaiiensis* off the North West Slope in August and 70–80 mm ML juveniles in April, suggesting hatching at different times of the year. From plankton surveys and collection of mature females in North West Slope waters, Dunning (1988a) concluded that *N. hawaiiensis* spawned year-round because paralarvae and juveniles were captured in September and October, and mature females were captured in February, April, August, and late September.

## Statolith zones

The origin of the opaque and translucent zones within the statolith microstructure of some of the *N.*

*hawaiiensis* specimens is unclear. Similar zones that occur in statoliths of the deepwater onychoteuthid *Moroteuthis ingens* might be related to a habitat shift from a pelagic to demersal environment (Jackson, 1993b). The number of increments in the opaque zone was similar for the two species. No zonation was observed in any of 43 statoliths examined from *Nototodarus sloanii* in New Zealand (senior author's personal observ.) which is predominantly a pelagic species. *Nototodarus hawaiiensis* adults are trawled day and night on the seafloor and might be demersal. Pelagic tows at discrete depths would be useful for establishing the ontogenetic descent in this species.

### Age and life span of *Nototodarus hawaiiensis*

The deepwater habitat of *N. hawaiiensis* may prevent validation of statolith increment periodicity because of the difficulty of carrying out experiments on live individuals from this habitat. However, some evidence is available on the periodicity of statolith increments in other ommastrephids. Experimental maintenance with chemical markers has shown that statolith increments are laid down daily in the temperate north Atlantic *Illex illecebrosus* (Dawe et al., 1985; Hurley et al., 1985) and in *Todarodes pacificus* in the north Pacific (Nakamura and Sakurai, 1990, 1991). Furthermore, increment counts on successive cohorts suggest that statolith increments are laid down daily in both *Illex argentinus* in the south Atlantic (Uozumi and Shiba, 1993) and in *Nototodarus sloanii* in southern New Zealand waters (Uozumi and Ohara, 1993). We therefore assume that statolith increment periodicity is also daily in *N. hawaiiensis*.

If the assumption of daily periodicity in increment formation for *N. hawaiiensis* is correct, this suggests that the squid matures early and has a short life span. The species reaches maturity in less than 200 d off the Australian North West Slope, considerably earlier than its conspecifics *Nototodarus gouldi* and *N. sloanii* in New Zealand waters, which live for about a year (Uozumi and Ohara; 1993, Uozumi et al., 1995). However, specimens obtained from trawls in this study were smaller than the maximum size recorded for *N. hawaiiensis*. The largest individuals recorded from Australia were 248 mm ML for a female captured off the Northwest Shelf, and 215 mm ML for a male off southern Queensland (Dunning, 1988b; Dunning and Förch, in press). Based on the regressions in Figure 2, and assuming linear growth throughout the life span, the total number of increments even in these larger individuals would be less than 250. However, *N. hawaiiensis* females have been reported to reach 290 mm ML in the western Indian Ocean and 318 mm ML in the southeastern Pacific. In contrast, individuals of *N. hawaiiensis* are much smaller in the Hawaiian and Philippine waters, with maximum sizes of 180 mm and 160 mm ML, respectively (Dunning and Förch, in press). There thus appear to be regional differences in maximum size (and possibly age) attained by this species.

On the basis of statolith analysis of *N. hawaiiensis* in Australia, this species may complete its life cycle in less than one year. It has a growth rate and life span comparable to tropical Australian shallow-water loliginids, which complete their life cycle in less than 200 d (Jackson, 1990; Jackson and Choat; 1992; Jackson and Yeatman 1996). *Nototodarus hawaiiensis* spends a considerable proportion of its adult life in deeper, cooler waters compared with the loliginids. However, many oceanic squids spend a proportion of their early life phase in the epipelagic zone (Roper and Young, 1975; Vecchione, 1987; Bigelow, 1992) Therefore, a considerable proportion of the life span of *N. hawaiiensis* is probably spent in warmer, epipelagic waters. The youngest individual captured at depth in this study was 49 d, which suggests that perhaps the first 50 days (approximately 25% of the life span) might be spent in the epipelagic zone. Forsythe (1993) proposed a model of squid growth that predicted that increased temperature during a squid's early growth phase can dramatically increase its growth rate, resulting in a much larger adult size. This model has recently been validated by seasonal growth data for *Lolliguncula brevis* (Jackson et al., 1997). *Nototodarus hawaiiensis* may therefore reach a larger size more quickly than if it spent most of its life span in cooler waters at depth.

The statolith analysis of *N. hawaiiensis* on the North West Shelf of Australia suggests a much shorter life span than that of other ommastrephids in tropical waters, e.g. *Todarodes angolensis* in the northern Benguela upwelling (Villanueva, 1992) and *Sthenoteuthis pteropus* in the tropical Atlantic (Arkhipkin and Mikheev, 1992) which have estimated life spans of around one year.

## Acknowledgments

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## Literature cited

**Arkhipkin, A. I.**
**1993.** Age, growth, stock structure and migratory rate of pre-spawning short-finned squid *Illex argentinus* based on

statolith ageing investigations. Fisheries Res. 16: 313–338.

**1996.** Geographical variation in growth and maturation of the squid *Illex coindetii* (Oegopsida, Ommastrephidae) off the north-west African coast. J. Mar. Biol. Assoc. U.K. 76:1091–1106.

**Arkhipkin, A. I., and A. Mikheev.**

**1992.** Age and growth of the squid *Sthenoteuthis pteropus* (Oegopsida: Ommastrephidae) from the Central-East Atlantic. J. Exp. Mar. Biol. Ecol. 163:261–276.

**Bigelow, K. A.**

**1992.** Age and growth in paralarvae of the mesopelagic squid *Abralia trigonura* based on daily growth increments in statoliths. Mar. Ecol. Prog. Ser. 82:31–40.

**Collins, M. A., G. M. Burnell, and P. G. Rodhouse.**

**1995.** Recruitment, maturation, and spawning of *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Irish waters. ICES J. Mar. Sci. 52:127–137.

**Bower, J. R.**

**1996.** Estimated paralarval drift and inferred hatching sites for *Ommastrephes bartramii* (Cephalopoda: Ommastrephidae) near the Hawaiian Archipelago. Fish. Bull. 94:398–411.

**Dawe, E. G., and P. C. Beck.**

**1997.** Population structure, growth, and sexual maturation of short-finned squid (*Illex illecebrosus*) at Newfoundland. Can. J. Fish. Aquat. Sci. 54:137–146.

**Dawe, E. G., R. K. O'Dor, P. H. Odense, and G. V. Hurley.**

**1985.** Validation and application of an ageing technique for short-finned squid (*Illex illecebrosus*). J. Northwest Atl. Fish. Sci. 6:07–116.

**Dunning, M. C.**

**1988a.** Distribution and comparative life history studies of deepwater squid of the family Ommastephidae in Australasian waters. Ph.D diss., Univ. Queensland, Queensland, 288 p.

**1988b.** First records of *Nototodarus hawaiiensis* (Berry, 1912) (Cephalopoda, Ommastrephidae) from Northern Australia. Mem. Mus. Vict. 49:59–168.

**Dunning, M. C., and E. C. Förch.**

**In press.** A review of the systematics, distribution and biology of arrow squids of the genus *Nototodarus* Pfeffer, 1912 (Cephalopoda, Ommastrephidae). Smith. Cont. Zool. 586.

**Forsythe, J. W.**

**1993.** A working hypothesis on how seasonal temperate change may impact the field growth of young cephalopods. *In* T. Okutani, R. K. O'Dor, and T. Kubodera (eds.), Recent advances in cephalopod fisheries biology, 133–143. Tokai Univ. Press, Tokyo.

**Guerra, A., and B. G. Castro.**

**1994.** Reproductive-somatic relationships in *Loligo gahi* (Cephalopoda: Loliginidae) from the Falkland Islands. Antarctic Science 6:175–178.

**Hurley, G. V., P. H. Odense, R. K. O'Dor, and E. G. Dawe.**

**1985.** Strontium labelling for verifying daily growth increments in the statolith of the short finned squid (*Illex illecebrosus*). Can. J. Fish. Aquat. Sci. 42:380–383.

**Ikeda, Y., Y. Sakurai, and K. Shimazaki.**

**1991.** Development of female reproductive organs during sexual maturation in the Japanese common squid *Todarodes pacificus*. Nippon Suisan Gakkaishi 57:2243–2247.

**Jackson, G. D.**

**1990.** Age and growth of the tropical near shore loliginid squid *Sepioteuthis lessoniana* determined from statolith growth ring analysis. Fish. Bull. 88:113–118.

**1993a.** Seasonal variation in reproductive investment in the tropical loliginid squid *Loligo chinensis* and the small tropical sepioid *Idiosepius pygmaeus*. Fish Bull. 91: 260–270.

**1993b.** Growth zones within the statolith microstructure of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae): evidence for a habitat shift? Can. J. Fish. Aquat. Sci. 50:2366–2374.

**1994.** Application and future potential of statolith increment analysis in squids and sepioids. Can. J. Fish. Aquat. Sci. 51:2612–2625.

**1997.** Age, growth and maturation of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. Polar Biol. 17:268–274.

**Jackson, G. D., J. Forsythe, R. F. Hixon, and R. T. Hanlon.**

**1997.** Age, growth and maturation of *Lolliguncula brevis* (Cephalopoda: loliginidae) in the Northwestern Gulf of Mexico with a comparison of length-frequency vs. statolith age analysis. Can. J. Fish. Aquat. Sci. 54:2907–2919.

**Jackson, G. D., and J. H. Choat.**

**1992.** Growth in tropical cephalopods: an analysis based on statolith microstructure. Can. J. Fish. Aquat. Sci. 49:218–228.

**Jackson, G. D., and C. C. Lu.**

**1994.** The statolith microstructure of seven species of antarctic squid captured in Prydz Bay, Antarctica. Antarctic. Sci. 6:195–200.

**Jackson, G. D., and P. V. Mladenov.**

**1994.** Terminal spawning in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae). J. Zool. Lond. 234:189–201.

**Jackson, G. D., and J. Yeatman.**

**1996.** Variation in size and age-at-maturity in *Photololigo* (Mollusca: Cephalopoda) from the North West Shelf of Australia. Fish. Bull. 94:59–65.

**Lipinski, M. R.**

**1981.** Statoliths as a possible tool for squid age determination. Bull. Acad. Pol. Sci. (Sci. Biol.). 28:569–82.

**Lipinski, M. R., E. E. Dawe and Y. Natsukari.**

**1991.** Introduction. *In* P. Jerev, Rs. Ragonese, and S.Von Boletzky (eds.), Squid age determination using statoliths, p. 77–81. Proceedings of an international workshop, 9–14 Oct. 1989, Mazara del Vallo, Italy. N.T.R.-I.T.P.P. Special Publication 1.

**Nakamura, Y., and Y. Sakurai.**

**1990.** On the daily formation of growth increments in the statoliths of Japanese common squid, *Todarodes pacificus*. Bull. Hokkaido Nat. Fish. Res. Inst. 54:1–7.

**1991.** Validation of daily growth increments in statoliths of Japanese common squid *Todarodes pacificus*. Nippon Suisan Gakkaishi 57:2007–2011.

**1993.** Age determination from daily growth increments in statoliths of some groups of the Japanese common squid *Todarodes pacificus*. *In* T. Okutani, R. K.O'Dor, and T. Kubodera (eds.), Recent advances in cephalopod fisheries biology: 337–342. Tokai Univ. Press, Tokyo.

**Rodhouse, P. G., and E. M. C. Hatfield.**

**1990.** Dynamics of growth and maturation in the cephalopod *Illex argentinus* de Castellanos, 1960 (Teuthoidea: Ommastrephidae). Philo. Trans. R. Soc. Lond. B Biol. Sci. 329:229–241.

**Rodhouse, P. G., K. Robinson, S. B. Gajdatsy, H. I. Daly, and M. S. J. Ashmore.**

**1994.** Growth, age structure and environmental history in the cephalopod *Martialia hyadesi* (Teuthoidea: Ommastrephidaw) at the Antarctic Polar Frontal Zone and on the Patagonian Shelf Edge. Antarct. Sci. 6:259–267.

**Roper, C. F. E., M. J. Sweeney, and C. E. Nauen.**
**1984.** Cephalopods of the world: an annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. 125(3), 277 p.

**Roper, C. F. E., and R. E. Young.**
**1975.** Vertical distribution of pelagic cephalopods. Smithsonian Cont. Zool. 209, 51 p.

**Uozumi, Y., S. Koshida, and S. Kotoda.**
**1995.** Maturation of arrow squids. (*Nototodarus gouldi* and *N. sloanii*) with age in New Zealand waters. Fish. Sci. 61:559–565.

**Uozumi, Y., and H. Ohara.**
**1993.** Growth and age composition of *Nototodarus sloanii* (Cephalopoda: Oegopsida) based on daily increment counts in statoliths. Nippon Suisan Gakkaishi 59:1469–1477.

**Uozumi, Y., and C. Shiba.**
**1993.** Growth and age composition of *Illex argentinus* (Cephalopoda: Oegopsida) based on daily increment counts in statoliths. *In* T. Okutani, R. K. O'Dor and T. Kubodera (eds.), Recent advances in cephalopod fisheries biology, 591–605. Tokai Univ. Press, Tokyo.

**Vecchione, M.**
**1987.** Juvenile ecology. *In* P.R. Boyle (ed.) Cephalopod life cycles, vol. II, p. 61–80. Academic Press, London.

**Villanueva, R.**
**1992.** Interannual growth differences in the oceanic squid *Todarodes angolensis* Adam in the northern Benguela upwelling system, based on statolith growth increment analysis. J. Exp. Mar. Biol. Ecol. 159:157–177.

**Voss, G. L.**
**1962.** Six new species and two new subspecies of cephalopods from the Philippine Islands. Proc. Biol. Soc. Wash. 75:169–176.

**Wadley, V. A.**
**1990.** Squid from the west and North West Slope trawl fishery. CSIRO Australia, 12 p.
**1993.** Cephalopods from demersal trawling on Australia's North West Slope. *In* T. Okutani, R.K.O'Dor and T. Kubodera (eds.), Recent advances in cephalopod fisheries biology, p. 607–617. Tokai Univ. Press, Tokyo.

**Abstract.**—Pigmentation patterns, meristic characters, morphometric measurements, and head spination were recorded and illustrated for the developmental series of larval and pelagic juvenile (10.0–27.7 mm SL) grass rockfish, *Sebastes rastrelliger*. Larvae were identified by the presence of strong dorsal and postanal ventral midline pigment and by the presence of pigment on the lateral midline. Juveniles became highly pigmented over most of their body and fin surfaces at a length of 27.7 mm. Pigment patterns were sufficiently distinct and consistent to differentiate larval and juvenile *S. rastrelliger* from other *Sebastes* species occurring off central California. Otolith characters were also useful in identification of this species. Larval and juvenile *S. rastrelliger* grew at a rate of 0.36 mm/day, similar to the growth rates observed in other species of *Sebastes*.

# Description of pelagic larval and juvenile grass rockfish, *Sebastes rastrelliger* (family Scorpaenidae), with an examination of age and growth

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Rockfishes (*Sebastes* spp.) support important commercial and recreational fisheries in the northeastern Pacific Ocean. During 1995, they accounted for approximately 12.6% of the commercial groundfish catch in weight (PFMC[1]) and 19.4% of all species in the recreational landings off California, Oregon, and Washington (Witzig et al., 1992). Management of rockfishes has been difficult owing to the large number of species within this genus. Sixty-one species of rockfish are reported from California alone (Eschmeyer et al., 1983). The need to identify and separate adult rockfish landed by the fishery has long been recognized (Phillips, 1964; Chen, 1971). Larvae and juveniles are far more difficult to differentiate than adults, but the need for their accurate identification is growing with their use in biomass estimates and recruitment studies (Moser and Butler, 1987; Hunter and Lo, 1993; Ralston et al.[2]). Here we provide means to identify the larvae and juveniles of grass rockfish, *S. rastrelliger*.

*Sebastes rastrelliger* is a thick-bodied, medium-size (maximum size, 56 cm), bottom-dwelling rockfish that inhabits shallow, rocky areas (maximum depth, 46 m) from central Baja California to Yaquina Bay, Oregon (Eschmeyer et al., 1983). This species accounts for less than 1% of the commercial groundfish catch by weight (Pearson[3]). However, in recent years landings have increased from 1.5 metric tons (t) in 1991 to 52 t in 1995. This may be attributed to a relatively new live fish fishery for this species in southern California (Love, 1996). Additionally, *Sebastes rastrelliger* is also an important part of the nearshore recreational catch (Love, 1996).

Manuscript accepted 11 February 1998.
Fish. Bull. 96:788–796 (1998).

[1] PFMC (Pacific Fishery Management Council). 1996. Status of the Pacific coast groundfish fishery through 1994 and recommended acceptable biological catches for 1997. Pacific Fishery Management Council, Portland, OR, 168 p.

[2] Ralston, S., J. R. Bence, M. B. Eldridge, and W. H. Lenarz. 1993. Estimating the spawning biomass of shortbelly rockfish (*Sebastes jordani*) in the region of Pioneer and Ascension Canyons using a larval production method, 32 p. Southwest Fisheries Science Center, Natl. Mar. Fish. Serv., NOAA, 3150 Paradise Drive, Tiburon, CA 94920.

[3] Pearson, D. E. 1994. Southwest Fish. Sci. Center, Natl. Mar. Fish. Serv., 3150 Paradise Drive, Tiburon, CA, 94920. Personal commun., 1997.

Only a few studies have been conducted on the development of *S. rastrelliger*. Moreno (1990) described the pigmentation patterns of laboratory-reared larvae up to 53 days old and 7.2 mm in length. Laroche[4] described the pigmentation of a 27.0-mm individual. The purpose of this study is to describe the development of *S. rastrelliger* from larvae to the pelagic juvenile stage and to examine the age and growth of larvae and juveniles.

## Methods

Specimens of pelagic larval and juvenile *S. rastrelliger* were obtained from research cruises conducted aboard the NOAA RV *David Starr Jordan*. Specimens were collected with a 26 × 26-m midwater trawl (12.7-mm stretched mesh codend liner). Surveys were conducted in the spring of 1990, 1992–94, and 1996. Specimens from midwater trawls were frozen for later analysis. All samples were collected off central California between Cypress Point (36°35'N) and Salt Point (38°35'N).

We examined pigmentation patterns and physical characteristics of 18 *S. rastrelliger* larvae and pelagic juveniles. Standard length (SL) was measured for each individual, and sizes ranged from 10.0 mm to 27.7 mm. Specimens greater than 19.9 mm were identified by meristic characters (Chen, 1986; Matarese et al., 1989; Moreland and Reilly, 1991; and Laroche[4]), and pigment patterns were recorded. Specimens less than 20 mm were initially identified from pigment patterns developed from a size series based on the patterns observed by Moreno (1990) and the pigment patterns of the smallest, positively identifiable individuals with complete meristic characters. Whenever possible, dorsal, anal, and pectoral-fin ray counts and the number of gill rakers on the first gill arch were recorded and subsequently used in identifications. Gill-raker counts were obtained only from fish larger than 15 mm in length.

Snout to anus length, head length, snout length, eye diameter, body depth at the pectoral-fin base, body depth at anus, and pectoral-fin length were measured on 17 specimens ranging from 10.0 to 27.7 mm. Terminology for morphometrics follows Richardson and Laroche (1979).

Fifteen specimens (ranging from 10.0 to 27.7 mm) were stained with alizarin red-S and examined for head spination. Terminology for head spination follows Richardson and Laroche (1979).

Otoliths were removed from 16 larvae and juveniles (10.0 to 27.7 mm) and ages were determined following the procedures in Laidig et al. (1991). Additionally, extrusion check radius was measured following the procedures in Laidig and Ralston (1995). Transformation from the larval stage to the juvenile stage was ascertained by the presence of secondary primordia in the otolith (Laidig et al., 1991).

## Results

### General development

At 10.0 mm, *S. rastrelliger* larvae had already undergone flexion, and a full complement of adult fins rays was present (13 dorsal, 6 anal, and 19 pectoral-fin rays), although the spinous dorsal rays were not quite fully developed (Table 1; Fig. 1C). Meristic counts were similar to those reported by Laroche[4] and Moreland and Reilly (1991). In our sample, the earliest signs of transformation were observed in a specimen 20.9 mm in length, and all specimens 27.7 mm or larger had completed transformation. Lateral line pores were present only in the largest individual; therefore a full complement was never observed during this study.

### Morphometrics

Notable changes in body proportion for *S. rastrelliger* occurred between lengths of 10.0 and 13.8 mm and during juvenile transformation (Table 2). At 10.0 mm, larvae had large heads in relation to their body size, whereas larger larvae became more elongate and thus decreased the proportion of head size in relation to body

**Table 1**

Frequency of occurrence of dorsal, anal, and pectoral-fin ray, and gill-raker counts in larval and juvenile grass rockfish, *Sebastes rastrelliger*.

| Character | Count | Frequency of occurrence | Percent occurrence |
|---|---|---|---|
| Dorsal-fin rays | 12 | 2 | 11.1 |
| | 13 | 16 | 88.9 |
| Anal-fin rays | 6 | 18 | 100 |
| Pectoral-fin rays | 18 | 2 | 11.8 |
| | 19 | 15 | 88.2 |
| Gill rakers | 21 | 2 | 16.7 |
| | 22 | 6 | 50.0 |
| | 23 | 3 | 25.0 |
| | 24 | 1 | 8.3 |

[4] Laroche, W. A. 1987. Guide to larval and juvenile rockfishes (*Sebastes*) of North America. Box 216, Enosburg Falls, VT 05450. Unpubl. manuscript, 311 p.

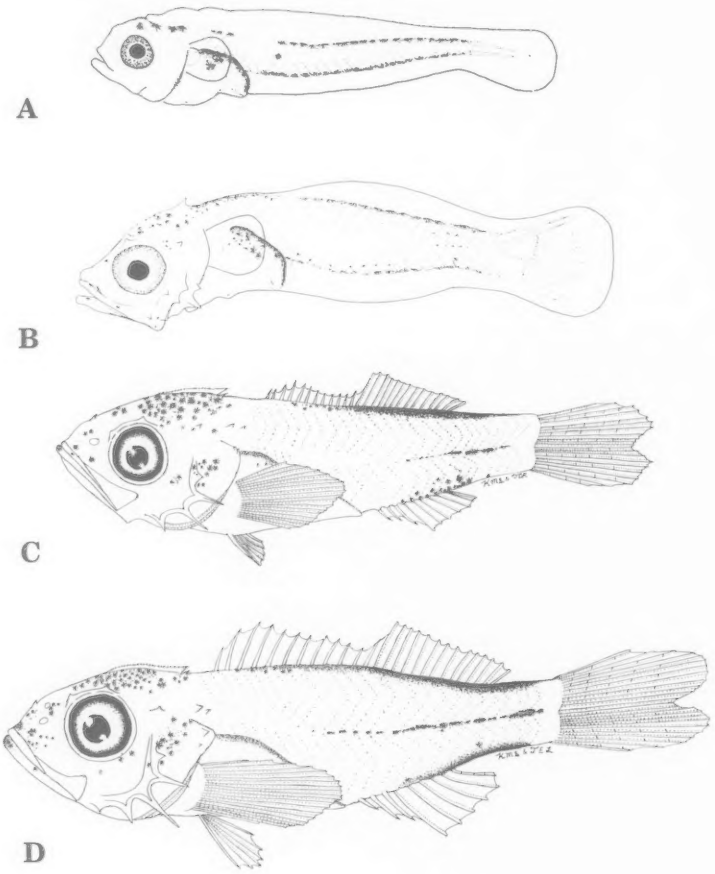


**Figure 1**

Developmental series of grass rockfish, *Sebastes rastrelliger*. (**A**) 4.6 mm SL yolksac preflexion larva; (**B**) 7.2 mm SL flexion larva; (**C**) 10.0 mm SL larva; (**D**) 13.8 mm SL larva; (**E**) 18.3 mm SL larva; (**F**) 21.6 mm SL transforming larva; (**G**) 27.7 mm SL pelagic juvenile. Illustrations A and B (4.6 and 7.2 mm SL, respectively) from Moreno, 1993.

length. After transformation, the fish became more thick-bodied, as evidenced by the increase in body depth at the pectoral-fin ray base and at the anus.

### Head spination

Several head spines had already developed by a length of 10.0 mm (Table 3). The nasal, postocular, parietal, nuchal, pterotic, inferior posttemporal, supracleithral, opercular, and preopercular were all well formed, whereas the 1st inferior and 1st superior infraorbital were barely perceptible at 10.0 mm. By 13.8 mm, the preocular, 2nd inferior infraorbital, and the superior posttemporal were developed. At 18.8 mm, the 4th superior infraorbital was formed. The tympanic formed at 19.7 mm. The pterotic became overgrown and embedded by 21.6 mm. The supraocular, coronal, 3rd inferior infraorbital, and 2nd and 3rd superior infraorbital were not apparent at any length examined.

### Body pigmentation

A distinct pattern of heavy dorsal and postanal ventral midline pigment, along with strong lateral midline pigment, was characteristic at a length of 10.0

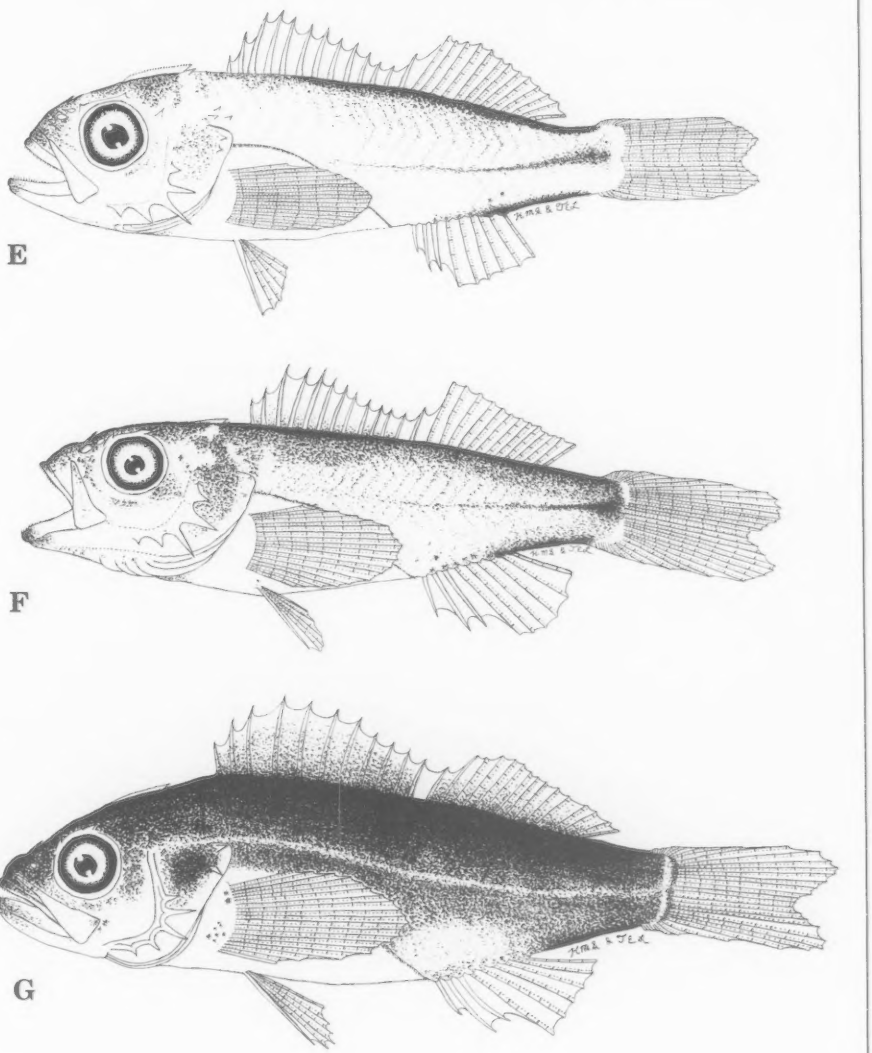


**Figure 1 (continued)**

mm (Fig. 1C; Table 4). Pigment was also evident along the anterior tip of the lower jaw, on the snout anterior to the eye orbit, on the top of the cranium, on the operculum, and along the dorsal and posterior margin of the gut cavity.

Pigmentation patterns at a length of 13.8 mm were similar to, but more intensely developed than, those at 10.0 mm (Fig. 1D). Pigment advanced in the anterior direction along the postanal ventral and lateral midline, and to a lesser degree along the dorsal midline. In addition, pigment along the posterior-ventral portion of the eye orbit had begun to form.

At a length of 18.3 mm, the dorsal half of the fish had become highly pigmented (Fig. 1E). Nape pigment had developed and merged with the dorsal midline pigment, forming a continuous band from the parietal and nuchal spines to the beginning of the caudal fin at this size. Snout and cranial pigment also had merged to form a solid line of pigment from the upper jaw to the parietal and nuchal spines at a

**Table 2**

Morphometric measurements (in mm) of grass rockfish, *Sebastes rastrelliger*.

| SL (mm) | Snout–anus length | Head length | Snout length | Eye diameter | Body depth at pectoral-fin ray base | Body depth at anus | Pectoral fin length |
|---|---|---|---|---|---|---|---|
| 10.0 | 6.4 | 3.7 | 1.2 | 1.2 | 2.9 | 2.4 | 1.7 |
| 13.8 | 7.9 | 4.5 | 1.5 | 1.6 | 3.4 | 3.1 | 2.9 |
| 16.7 | 8.4 | 5.0 | 1.7 | 1.8 | 3.8 | 3.3 | 3.4 |
| 16.7 | 8.4 | 5.0 | 1.7 | 1.8 | 3.8 | 3.3 | 3.4 |
| 16.8 | 8.9 | 5.4 | 1.8 | 1.9 | 3.8 | 3.4 | 3.4 |
| 17.4 | 10.2 | 5.8 | 2.1 | 1.9 | 3.9 | 3.3 | 3.6 |
| 18.0 | 10.5 | 5.9 | 2.2 | 2.0 | 4.3 | 3.4 | 3.8 |
| 18.3 | 10.5 | 6.2 | 2.2 | 2.0 | 4.3 | 3.9 | 3.7 |
| 18.8 | 10.8 | 6.3 | 2.3 | 2.1 | 4.5 | 3.9 | 3.8 |
| 19.2 | 10.9 | 6.5 | 2.4 | 2.1 | 4.6 | 3.7 | 4.0 |
| 19.3 | 11.1 | 6.8 | 2.5 | 2.2 | 4.6 | 3.8 | 4.2 |
| 19.7 | 11.4 | 6.9 | 2.5 | 2.2 | 4.8 | 3.9 | 4.3 |
| 20.9 | 11.7 | 7.2 | 2.7 | 2.3 | 5.1 | 4.4 | 4.5 |
| 21.6 | 12.8 | 7.5 | 2.9 | 2.4 | 5.9 | 5.2 | 4.8 |
| 22.7 | 13.7 | 7.9 | 3.0 | 2.5 | 6.2 | 5.5 | 5.2 |
| 24.3 | 14.0 | 8.7 | 2.5 | 2.7 | 6.7 | 5.7 | 5.8 |
| 27.7 | 15.9 | 9.0 | 2.6 | 2.9 | 7.6 | 6.5 | 6.7 |

length of 18.3 mm. An unpigmented section was conspicuous between the nape and cranial regions. Pigment on the flanks of the dorsal body intensified, especially in between the myomeres. Lateral midline pigment extended from the caudal peduncle to the gut cavity. Pigment on the lateral midline near the caudal peduncle coalesced into a large pigmented area, which decreased in intensity anteriorly. Much of the dorsal half of the operculum was covered with pigment.

At 18.3 mm, the ventral half of the body remained mostly unpigmented, except for the presence of a series of melanophores on the ventral surface anterior to the gut cavity and between the branchiostegals (Fig. 1E). Pigment at the anterior tip of the lower jaw remained similar in intensity to that of the 13.8-mm specimen. Postanal ventral midline pigment became reduced, especially at the base of the anal fin. Pigment was also absent between the ventral myomeres.

By 21.6 mm, pigmentation increased over the entire body surface (Fig. 1F). The dorsal midline pigment merged with the nape, head, snout, and anterior tip of the upper jaw pigment to form a solid line along the dorsal surface of the body from the mouth to the caudal fin. Pigment formed on the ventral half of the membranes of the spinous dorsal fin. Pigment increased around the ventral surface of the eye orbit. Nape, head, and operculum pigment merged to form a vertical bar from the operculum to the dorsal midline, except for a small unpigmented area near the nuchal and parietal spines. Hypural pigment began to form, and the caudal peduncle became highly pigmented. The ventral surface of the body remained only lightly pigmented. A few pigment spots were observed at the base of the pelvic fin.

Juvenile *S. rastrelliger* became highly pigmented throughout most of their body surface by 27.7 mm (Fig. 1G). The entire dorsal surface of the body was completely pigmented. Pigment also covered much of the ventral surface of the body. The gut cavity, the ventral surface of the head, and the area just above the anal fin still remained largely unpigmented. Some pigment was observed at the base of the pectoral fin. A line of light pigment occurred along the lateral midline. Hypural pigment became intense, but an area of decreased pigment was evident just anterior to the hypural area. The spinous and soft dorsal fins were highly pigmented, except for the membrane between spine 11 and 12 which was completely devoid of pigment. A few pigment spots occurred on the anal and caudal fins.

## Otolith examination

A linear model provided a good fit for the relationship of total otolith radius versus SL ($r^2$=0.94)(Fig. 2). The growth rate of *S. rastrelliger* was estimated by a linear model fitted to the relationship of SL and age (coefficient of determination, $r^2$=0.88)(Fig. 3). This model estimated a growth rate of 0.36 mm/day and an extrusion SL of 0.15 mm. The extrusion check radius for *S. rastrelliger* ranged from 13.3 to 14.6 µm, averaging 14.0 µm (SD=0.39). Secondary primordia

**Table 3**

Development of head spines in grass rockfish, *Sebastes rastrelliger*. "1" means spine present and "0" means spine absent.

| | Standard length (mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spines | 10.0 | 13.8 | 16.7 | 17.4 | 18.0 | 18.3 | 18.8 | 19.2 | 19.3 | 19.7 | 20.9 | 21.6 | 22.7 | 24.3 | 27.7 |
| Nasal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Preocular | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Supraocular | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Postocular | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Coronal | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tympanic | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Parietal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Nuchal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Pterotic | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| Posttemporals | | | | | | | | | | | | | | | |
| Superior | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Inferior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Supracleithral | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Opercular | | | | | | | | | | | | | | | |
| Superior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Inferior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Preoperculars | | | | | | | | | | | | | | | |
| 1st anterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2nd anterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3rd anterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1st posterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2nd posterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3rd posterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 4th posterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5th posterior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Infraorbitals | | | | | | | | | | | | | | | |
| 1st inferior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2nd inferior | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3rd inferior | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1st superior | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2nd superior | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3rd superior | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4th superior | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

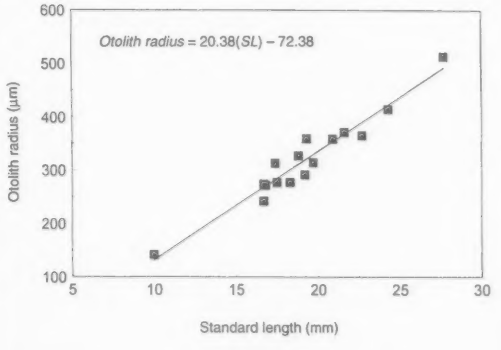


**Figure 2**

Plot of total otolith radius and standard length of grass rockfish, *Sebastes rastrelliger* ($n$=16). Solid line indicates predicated values from linear model.

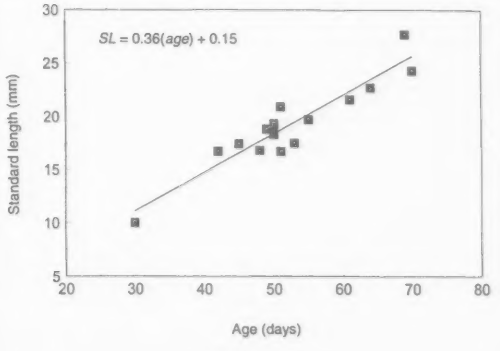


**Figure 3**

Plot of standard length and age of grass rockfish, *Sebastes rastrelliger* ($n$=16). Solid line indicates predicated values from linear model.

**Table 4**

Pigment occurrence at various pigment loci for grass rockfish, *Sebastes rastrelliger*. SL = standard length in mm. Definitions of pigment loci are given below. 0.0 = no pigment, 1.0 = some pigment present, and 2.0 = area heavily pigmented. LJ = anterior tip of the lower jaw, EYE = posterior-ventral edge of the eye orbit, HEAD = cranial surface, FACE = dorsal surface anterior to the eyes, OPER = operculum, CHK = radiating cheek bars, NAPE = nape pigment, DORS = dorsal body surface, VENT = ventral body surface, MID = along the lateral midline, HYP = hypural region, DFIN = spinous dorsal fin, AFIN = anal fin, PEC = blade of the pectoral fin.

| SL | LJ | EYE | HEAD | FACE | OPER | CHK | NAPE | DORS | VENT | MID | HYP | DFIN | AFIN | PEC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 0.0 | 1.0 | 2.0 | 2.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13.8 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 1.0 | 2.0 | 2.0 | 2.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| 16.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16.8 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 17.4 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 17.5 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18.3 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 18.8 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 1.0 | 0.0 | 0.0 |
| 19.2 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19.3 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 1.0 | 0.0 | 0.0 |
| 19.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20.9 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 1.0 | 0.0 | 0.0 |
| 21.6 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 0.0 | 0.0 |
| 22.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 0.0 | 0.0 |
| 24.3 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 2.0 | 0.0 | 0.0 |
| 27.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.0 | 0.0 |

first appeared in the otoliths of the 20.9-mm specimen and was subsequently observed in the otoliths of all specimens larger than 20.9 mm. By 27.7 mm, the otolith was completely encircled by secondary primordia, signaling the end of juvenile transformation.

## Discussion

*Sebastes rastrelliger* have developed a unique pigment pattern by 10 mm (Fig. 1C), which allows them to be distinguished from other *Sebastes* spp. At this size, the presence of dorsal, ventral, and lateral body midline pigment is distinctive for only three species (*S. dallii* [Moser and Butler, 1981], *S. rufus* [Moser and Butler, 1987], and *S. saxicola* [Laidig et al., 1996]) that co-occur with *S. rastrelliger* off California. All four species have anterior lower jaw, snout, and opercular pigments, which could add to the confusion between these species. One major difference in pigmentation between these species is that *S. dallii* and *S. rufus* have pectoral-fin pigment. We found no pectoral-fin pigmentation in *S. rastrelliger* larger than 10.0 mm, and Moreno (1990) found no pectoral-fin pigment for individuals smaller than 8 mm. *Sebastes saxicola* also lacks pectoral-fin pigment in fish smaller than 20 mm (Laidig et al., 1996). *Sebastes saxicola* and *S. rastrelliger* can exhibit similar pigmentation at 10 mm. This similarity occurs until approximately 18 mm, when *S. saxicola* develops hypural and dorsal fin pigment and when saddling along the dorsal surface eventually changes into a barred pattern. *Sebastes rastrelliger* was also found to have a relatively small pectoral fin for its SL (Table 2) in comparison with other *Sebastes* spp. (Moser et al., 1977; Richardson and Laroche, 1979; Laroche and Richardson, 1980, 1981; Sakuma and Laidig, 1995; Laidig et al., 1996)

Meristics can aid in differentiating *S. rastrelliger* from other species. The average counts of 13 dorsal, 6 anal, and 19 pectoral-fin rays (Table 1) are similar to those observed by Moreland and Reilly (1991) and Laroche.[4] Moreland and Reilly (1991) found this combination of fin-ray counts typical for only two species: *S. rastrelliger* and *S. babcocki*. *Sebastes babcocki* has a distinct barred pigmentation pattern and typical gill-raker counts of 30–31. *Sebastes rastrelliger* had no barred pattern and average gill-raker counts of 22. This count is lower than that observed by Moreland and Reilly (1991) (an average of 25) and Laroche[4] (an average of 24). Gill-raker counts may be low in our study because the gill rakers may not have fully developed in some of our specimens (Sakuma and Laidig, 1995). Therefore, the use of pigment patterns in conjunction with meristics should aid in the identification of *S. rastrelliger*.

Otolith characters can also be useful in separating *Sebastes rastrelliger* from some other *Sebastes* species. Laidig et al. (1996) showed that the extrusion check radius of *S. rastrelliger* (14.0 μm) was significantly different from those of *S. saxicola*, *S. maliger*, *S. atrovirens*, and the copper complex (*S. carnatus*, *S. caurinus*, and *S. chrysomelas*). It was not different from those of *S. auriculatus* or *S. semicinctus*. However, these latter two species have distinctly different meristic counts and pigmentation patterns. In addition, the extrusion check radii found by Laidig and Ralston (1995) for *S. paucispinis*, *S. flavidus*, *S. entomelas*, and *S. mystinus* were smaller (10.93–12.20 μm) than that for *S. rastrelliger*, whereas the extrusion check radii for *S. jordani* and *S. goodei* were much larger (15.15–16.96 μm).

Postflexion *Sebastes rastrelliger*, from 10.0 to 27.7 mm, grew at a rate of 0.36 mm/day. Moreno (1990) found that the growth rate for reared preflexion *S. rastrelliger* less than 8 mm was 0.07 mm/day. This slow growth rate may be attributed to laboratory rearing of the fish. However, slow growth rates have been shown for the first few weeks in *S. goodei* (Sakuma and Laidig, 1995), *S. saxicola* (Laidig et al., 1996), and *S. jordani* (Laidig et al., 1991). Sakuma and Laidig (1995) observed growth rates of 0.135 mm/day in *S. goodei* less than 40 days old whereas Woodbury and Ralston (1991) noted growth rates of 0.399–0.555 mm/day in specimens 35–170 days old. Laidig et al. (1996) observed growth rates of 0.125 mm/day in *S. saxicola* less than 40 days old, whereas larvae and juveniles older than 40 days exhibited increased growth rates of 0.367 mm/day. Laidig et al. (1991) determined that in *S. jordani* there were large fluctuations in growth rates associated with flexion, with relatively slow growth prior to flexion, almost no growth during flexion, and relatively rapid growth after flexion. Because the *S. rastrelliger* in this study had already undergone flexion, we expected a faster growth rate than that of smaller fish as recorded by Moreno (1990). For this reason, our growth curve does not accurately express the growth in the first few weeks of life; this lack of fit is consistent with the smaller than expected estimate of SL at extrusion (0.15 mm). Therefore, extrapolating growth rates for flexion and preflexion larvae from our model would not be advised.

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## Literature cited

**Chen, L.**
**1971.** Systematics, variation, distribution, and biology of rockfishes of the subgenus *Sebastomus* (Pisces, Scorpaenidae, *Sebastes*). Univ. California Press, Berkeley, CA, 115 p.
**1986.** Meristic variation in *Sebastes* (Scorpaenidae), with an analysis of character association and bilateral pattern and their significance in species association. U. S. Dep. Commer., NOAA Tech. Rep. NMFS 45, 17 p.

**Eschmeyer, W. N., E. S. Herald, and H. Hammann.**
**1983.** A field guide to Pacific coast fishes. Houghton Mifflin Company, Boston, MA, 336 p.

**Hunter, J. R., and N. C. -H. Lo.**
**1993.** Ichthyoplankton methods for estimating fish biomass introduction and terminology. Bull. Mar. Sci. 53:723–727.

**Laidig, T. E., and S. Ralston.**
**1995.** The potential use of otolith characters in identifying larval rockfish (*Sebastes* spp.). Fish. Bull. 93:166–171.

**Laidig, T. E., S. Ralston, and J. R. Bence.**
**1991.** Dynamics of growth in the early life history of shortbelly rockfish, *Sebastes jordani*. Fish. Bull. 89: 611–621.

**Laidig, T. E., K. M. Sakuma, and M. M. Nishimoto.**
**1996.** Description of pelagic larval and juvenile stripetail rockfish, *Sebastes saxicola* (family Scorpaenidae), with an examination of larval growth. Fish. Bull. 94:289–299.

**Laroche, W. A., and S. L. Richardson.**
**1980.** Development and occurrence of larvae and juveniles of the rockfishes *Sebastes flavidus* and *Sebastes melanops* (Scorpaenidae) off Oregon. Fish. Bull. 77(4):901–922.
**1981.** Development of larvae and juveniles of the rockfishes *Sebastes entomelas* and *S. zacentrus* (family Scorpaenidae) and occurrence off Oregon, with notes on head spines of *S. mystinus*, *S. flavidus*, and *S. melanops*. Fish. Bull.79(2): 231–257.

**Love, M.**
**1996.** Probably more than you wanted to know about the fishes of the Pacific coast. Really Big Press, Santa Barbara, CA, 381 p.

**Matarese, A. C., A. W. Kendall Jr., D. M. Blood, and B. M. Vinter.**
**1989.** Laboratory guide to early life history stages of northeast Pacific fishes. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 80, 652 p.

**Moreland, S. L., and C. A. Reilly.**
**1991.** Key to the juvenile rockfishes of central California. *In* T. E. Laidig and P. B. Adams (eds.), Methods used to identify pelagic juvenile rockfish (genus *Sebastes*) occurring along the coast of central California, p. 59–180. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM-NMFS-SWFSC-166, 180 p.

**Moreno, G.**
**1990.** Description of the larval stages of five northern California species of rockfishes (Family Scorpaenidae) from rearing studies. M.S. thesis, California State Univ., Stanislaus, CA, 68 p.
**1993.** Description of early larvae of four northern California species of rockfishes (Scorpaenidae: *Sebastes*) from rearing studies. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 116, 18 p.

**Moser, H. G., and J. L. Butler.**
**1981.** Description of reared larvae and early juveniles of the calico rockfish, *Sebastes dallii*. Calif. Coop. Oceanic. Fish. Invest. Rep. XXII:88–95.

**1987.** Descriptions of reared larvae of six species of *Sebastes*. *In* W. H. Lenarz, and D. R. Gunderson (eds.), Widow rockfish. Proceedings of a workshop, Tiburon, California, December 11–12, 1980, p. 19–29. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 48.

**Moser, H. G., E. H. Ahlstrom, and E. M. Sandknop.**

**1977.** Guide to the identification of scorpionfish larvae (Family Scorpaenidae) in the eastern Pacific with comparative notes on species of *Sebastes* and *Helicolenus* from other oceans. U.S. Dep. Commer., NOAA Tech. Rep. NMFS Circ. 402, 71 p.

**Phillips, J. B.**

**1964.** Life history studies on ten species of rockfish. Calif. Dep. Fish Game Fish Bull. 126, 70 p.

**Richardson, S. L., and W. A. Laroche.**

**1979.** Development and occurrence of larvae and juveniles of the rockfishes *Sebastes crameri*, *Sebastes pinniger*, and *Sebastes helvomaculatus* (Family Scorpaenidae) off Oregon. Fish. Bull. 77(1):1–46.

**Sakuma, K. M., and T. E. Laidig.**

**1995.** Description of larval and pelagic juvenile *Sebastes goodei* with an examination of larval growth. Fish. Bull. 93:720–730.

**Witzig, J. F., M. C. Holliday, R. J. Essig, and D. L. Sutherland.**

**1992.** Marine recreational fisheries statistics survey, Pacific coast, 1987–1989. National Marine Fisheries Service, Silver Springs, MD, 367 p.

**Woodbury, D. P., and S. Ralston.**

**1991.** Interannual variation in growth rates and back-calculated birthdate distributions of pelagic juvenile rockfishes (*Sebastes* spp.) off the central California coast. Fish. Bull. 89:523–533.

# Changes in the sex ratio and size at maturity of gag, *Mycteroperca microlepis*, from the Atlantic coast of the southeastern United States during 1976–1995*

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**Abstract.**–Gag, *Mycteroperca microlepis*, is a large, slow-growing, protogynous grouper that probably makes annual migrations to specific locations to aggregate for spawning. During 1976–82, male gag constituted 19.6% of the sexually mature individuals taken during fishery-dependent and fishery-independent sampling along the southeast coast of the United States. A similar percentage of males was found in the Gulf of Mexico from 1977 to 1980; however, males made up only 1.9% of the population in the Gulf of Mexico during 1992. To assess the current sex ratio of gag along the southeast U.S. coast, an emergency rule was enacted by the Department of Commerce in January 1995 that required commercial vessels from North Carolina to southeast Florida to land gag with gonads intact. Histological examination of 2613 gonads of sexually mature gag collected from 18 January through 18 April 1995 revealed that 5.5% of the gag from the southeast Atlantic were male. There was a weak trend indicating that females reached maturity at a smaller size in 1994–95 than in 1976–82. Very few transitional specimens were collected during the spawning season. Most transitional individuals (79%) were taken during April through June immediately after the 1995 spawning season. Gag in spawning condition were landed during December through mid-May by fishermen working offshore from North Carolina to southeast Florida. In addition, gag in spawning condition were taken during research cruises documenting the occurrence of spawning north of Florida (off South Carolina and Georgia at depths ranging from 49 to 91 m).

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Fish. Bull. 96:797–807 (1998).

Gag, *Mycteroperca microlepis*, is a large, slow-growing serranid associated with inshore-reef and shelf-break habitats in the western Atlantic from New York to Brazil and in the Gulf of Mexico (Smith, 1971; Huntsman, 1976; Hardy, 1978; Collins et al., 1987). Gag are protogynous and probably make annual late-winter migrations to specific locations to form spawning aggregations (Collins et al., 1987; Keener et al., 1988; Van Sant et al., 1994). Recent evidence indicates that males may be selectively removed because they are the largest and most aggressive individuals in a spawning aggregation and are the first to be taken by fishing gear (Gilmore and Jones, 1992). Effects of fishing on spawning aggregations in other grouper species have been found to be deleterious on population size, sex ratio, genetic diversity, and behavior of individuals (Nelson and Soulé, 1987; Smith et al., 1991; Carter et al., 1994; Coleman et al., 1996).

Histological examination of gonads of 498 gag collected throughout the year during 1976–82 along the southeast coast of the United States revealed that 84% were females, 15% males, and 1% were undergoing transition from female to male (Collins et al., 1987). Hood and Schlieder (1992) found similar sex ratios (14.0% male) for Gulf of Mexico gag collected from 1977 to

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1980. In both of these earlier studies, immature females were included in the sex ratio. In 1992, Coleman et al. (1996) determined that males made up a smaller percentage (1.9%) of the Gulf of Mexico adult (sexually mature) population than did males in the earlier study by Hood and Schlieder (1992). No current estimate of sex ratio is available for gag along the southeast coast of the United States.

In 1993, the South Atlantic Fishery Management Council (SAFMC) and the Florida Marine Fish Commission considered a closure for fishing of gag along the southeast coast during the gag spawning season to prevent the possibility that fishing on aggregations could severely decrease spawning output and affect sex ratios for future spawning seasons and generations (SAFMC, 1993). Much of the impetus for the proposed closure was the heavy fishing pressure exerted by recreational and commercial fishermen on spawning aggregations along the narrow Florida shelf. However, the SAFMC elected to take no action because it was unknown if gag spawned north of Florida and because the impacts of a spawning season closure for the entire southeast coast might be an unnecessary hardship on fishermen. In addition, no recent data were available on the sex ratio of gag along the southeast coast. Owing to the paucity of data on the current reproductive status of gag, the U.S. Department of Commerce followed recommendations from SAFMC and the National Marine Fisheries Service (NMFS) and enacted an emergency rule requiring all commercial vessels along the Atlantic coast of the southeastern United States to land gag in whole condition so that sex ratios and reproductive condition could be assessed. In this paper we compare current sex ratio and maturity data (1994–95) with historical information (1976–82). An additional objective of this work was to determine if gag spawn north of Florida.

## Methods

Gag reproductive data were collected during 1976–82 and 1994–95 by the Marine Resources Monitoring Assessment and Prediction (MARMAP) program at South Carolina Department of Natural Resources (SCDNR). Data collected from 1976 to 1982 were obtained from the commercial hook and line fishery or research cruises conducted by the MARMAP program. These data (1976–82) were published by Collins et al. (1987), but raw data were available to us for comparison with recent information. During 1994–95, the collection of most gag resulted from an emergency rule that required commercial fishermen along the southeast Atlantic coast to land all gag with gonads intact from 18 January through 18 April 1995. The rule was extended through 17 July 1995 but applied only to gag larger than 889 mm (TL). Upon reaching the dock, fishermen were instructed to contact a NMFS or state port sampler assigned to the region. The port samplers recorded date, port, approximate area of fishing, and total length (TL) and weight of specimens. All length measurements in our study refer to TL. Gonads were obtained from each specimen, packed in ice or preserved in formalin, and shipped to personnel of the MARMAP program. Additional samples were collected by MARMAP during 1994 and 1995 with chevron traps and hook-and-line gear at randomly chosen reef areas off the southeastern United States (Collins and Sedberry, 1991; Cuellar et al., 1996) and through port sampling efforts.

To assess sex and reproductive state, all gonads collected during 1994–95 were prepared for histological examination. The posterior portion of the gonad was removed and preserved in 10% formalin buffered with seawater. After a 2–6 week fixation, the tissue samples were transferred to 50% isopropanol, processed and vacuum infiltrated in a modular vacuum tissue processor, and blocked in paraffin. The resulting imbedded samples were sectioned at 7 µm, stained with double-strength Gill hematoxylin, and counter-stained with eosin-y. A similar technique was used for the 1976–82 samples (Collins et al., 1987).

Sex and reproductive stage were assessed by one reader according to histological criteria (Table 1). Sections from 100 randomly selected specimens were examined by a second reader early in the study to verify the assessments. Specimens with developing, ripe, spent, or resting gonads were considered to be sexually mature. For females, this definition of sexual maturity included specimens with oocyte development at or beyond the cortical granule (alveoli) stage and specimens with beta, gamma, or delta stages of atresia. To ensure that females were correctly assigned to the immature and resting categories, the length-frequency histogram for immature females was compared to the histograms for resting females and females with evidence of certain maturity (e.g. developing, ripe, or spent). If there was little or no overlap between the two histograms representing mature individuals and the histogram for immature females, it was assumed that immature and resting individuals were not being confused.

To produce a sex ratio for the adult portion of the population, only data for mature gag were included in analyses. Data from Collins et al. (1987) and Hood and Schlieder (1992) were analyzed again to determine a sex ratio based on mature gag so that the results of these studies and the present study could

**Table 1**

Histological criteria used to determine reproductive state in gag, *Mycteroperca microlepis* (based on Moe, 1969; Hunter and Macewicz, 1985; Hunter al. 1986; Collins et al., 1987; West, 1990; Ferreira, 1993; Shapiro et al., 1993a, 1993b).

| Reproductive state | Male | Female |
|---|---|---|
| Immature | No primary males found. "Immature males" in Moe (1969) considered late transitional because sex transition is not yet completed. | No evidence of atresia. In comparison to resting female, oogonia more abundant along the margin of lamellae, most previtellogenic oocytes <80 μm in diameter, area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles, lamellae are not as elongate, and ovarian wall is thinner. |
| Developing | Development of cysts containing primary and secondary spermatocytes through some accumulation of spermatozoa in lobular lumina and peripheral sinuses within gonadal wall. | Oocytes undergoing cortical granule (alveoli) formation through nucleus migration and partial coalescence of yolk globules. |
| Running ripe | Predominance of spermatozoa in lobules and peripheral sinuses. Little or no occurrence of spermatogenesis. | Completion of yolk coalescence and hydration in the most advanced oocytes. Zona radiata becomes thin. Postovulatory follicles sometimes present. |
| Spent | No spermatogenesis. Some residual spermatozoa in lobules and peripheral sinuses. | More than 50% of vitellogenic oocytes in alpha or beta stage of atresia. |
| Resting | Little or no spermatocyte development. Empty lobules and sinuses evident. | Traces of atresia. In comparison to immature female, oogonia less abundant along margin of lamellae, most previtellogenic oocytes >80 μm in diameter, area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongate and convoluted, and ovarian wall is thicker. |
| Developing, recent spawn | Not assessed. | Developing stage as described above plus presence of postovulatory follicles. |
| Mature specimen, state unknown | Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive state. | Mature, but inadequate quantity of tissue or postmortem histolysis prevents further assessment of reproductive state. |
| Transitional | | |
| Proliferation of spermatogonia through limited spermatogenesis within lamellae of resting ovary and development of peripheral sinuses in musculature of ovarian wall. | | |

be compared. The sex ratio for 1995 was based on mature individuals that were collected during the early portion of the emergency rule (18 January to 18 April 1995). Recent data were analyzed by region: North Carolina, South Carolina, Georgia, northern Florida, and southern Florida (south of New Smyrna Beach). Plots of reproductive seasonality were based on all individuals that were collected during 1994 and 1995. Females that possessed hydrated oocytes or postovulatory follicles were considered to be in spawning condition.

Data for all females collected during the two periods were used in size-at-maturity analyses. The probit procedure (SAS, 1990) was used to fit a gompit model with the Gompertz distribution function to maturity data in 5-cm-TL intervals. This procedure provided estimates of length at 50% maturity ($L_{50}$) and a comparison of size at maturity between periods. Analysis of variance (ANOVA) and the Duncan multiple range test were used to determine if there was a significant difference in the mean size of gag collected by month for mature individuals. ANOVA and the Duncan multiple range test were also used to determine if there was a significant difference in the monthly mean size of gag between 889 and 1050 mm TL to reduce the potential for bias that might result from selection of larger fishes during the latter part of the emergency rule. The results of all statistical tests were considered significant if $P$ was $<0.05$.

## Results

Histological examination of 2606 sexually mature gag (size range=517–1275 mm TL) taken during 18 January through 18 April 1995 revealed that there were significantly ($\chi^2$=66.852; $P$ <0.001) fewer males (5.5%) along the southeast coast of the United States than during 1976–82 (19.6%; Tables 2 and 3). There were no significant differences in the percentage of males captured in 1995 off North Carolina, South Carolina, Georgia, and southern Florida ($\chi^2$ =4.926; $P$>0.05; Table 4). However, the percentage of males taken in northern Florida (14.9%) was significantly greater than in all other regions combined ($\chi^2$ =133.160; $P$<0.001). Four collections accounted for over 35% of the males that were collected in northern Florida.

The size range of gag captured during 1994 and 1995 was similar to those caught from 1976 to 1982; however, there were some differences in size between periods for both sexes (Tables 3 and 5). Larger mature female gag were collected in 1994–95, although the mean lengths for the two periods were similar (1976–82: $\bar{x}$ TL=831 mm, SD=92; 1994–95: $\bar{x}$ TL = 832 mm, SD=82). The smallest mature female was

**Table 2**

Sex ratio of gag, *Mycteroperca microlepis*, (M=Male; F=Female; T=Transitional) taken during various studies in the northeast Gulf of Mexico and southeastern United States between 1976 and 1995. Sex ratios based on sexually mature individuals.

| Study | Dates | Area | No. of M | % M | No. of F | % F | No. of T | % T |
|---|---|---|---|---|---|---|---|---|
| This study | 1976–1982 | SE Atlantic | 104 | 19.6 | 419 | 78.9 | 8 | 1.5 |
| Hood and Schlieder (1992) | 1977–1980 | NE Gulf of Mexico | 125 | 15.6 | 659 | 82.5 | 15 | 1.9 |
| Coleman et al. (1996) | 1992 | NE Gulf of Mexico | 9 | 1.9 | 457 | 97.3 | 3 | 0.6 |
| This study | 18 Jan–18 April 1995[1] | SE Atlantic | 143 | 5.5 | 2468 | 94.4 | 2 | 0.1 |
| This study | 1994–1995 | SE Atlantic | 256 | 6.4 | 3720 | 92.7 | 39 | 0.9 |

[1] Period of emergency rule when fishermen were required to land all gag with gonads intact.

**Table 3**

Number of sexually mature gag, *Mycteroperca microlepis*, collected by size class during 1976–82 and 18 January to 18 April 1995 when fishermen were required to land all gag with intact gonads (TL=total length). Trans = transitional specimens.

| Size (mm TL) | 1976–1982 | | | 1995 | | |
|---|---|---|---|---|---|---|
| | Female | Male | Trans | Female | Male | Trans |
| 450–499 | — | — | — | — | — | — |
| 500–549 | 2 | — | — | 2 | — | — |
| 550–599 | 1 | — | — | 12 | — | — |
| 600–649 | 10 | — | — | 19 | — | — |
| 650–699 | 14 | — | — | 79 | — | — |
| 700–749 | 31 | — | — | 171 | — | — |
| 750–799 | 64 | 1 | 2 | 437 | — | |
| 800–849 | 94 | 1 | 1 | 709 | — | 1 |
| 850–899 | 79 | 1 | 2 | 580 | 5 | 1 |
| 900–949 | 42 | 4 | 1 | 271 | 3 | — |
| 950–999 | 7 | 13 | 1 | 99 | 26 | — |
| 1000–1049 | 13 | 14 | — | 48 | 48 | — |
| 1050–1099 | 5 | 27 | — | 27 | 36 | — |
| 1100–1149 | — | 14 | — | 6 | 13 | — |
| 1150–1199 | — | 2 | — | 1 | 7 | — |
| 1200–1249 | — | 1 | — | — | 5 | — |
| 1250–1299 | — | — | — | | — | — |
| No length | 47 | 25 | 1 | 7 | | |
| Total | 419 | 104 | 8 | 2468 | 143 | 2 |
| Percent | 78.9 | 19.6 | 1.5 | 94.4 | 5.5 | 0.1 |

**Table 4**

Number of sexually mature gag, *Mycteroperca microlepis*, females (F), males (M), and transitionals (T) taken in North Carolina, South Carolina, Georgia, northern Florida, and southern Florida during 18 January to 18 April 1995 when fishermen were required to land all gag with intact gonads (TL=total length).

| Size (mm TL) | North Carolina | | | South Carolina | | | Georgia | | | North Florida | | | South Florida | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | M | T | F | M | T | F | M | T | F | M | T | F | M | T |
| 450–499 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 500–549 | — | — | — | 2 | — | — | — | — | — | — | — | — | — | — | — |
| 550–599 | 1 | — | — | 10 | — | — | — | — | — | 1 | — | — | — | — | — |
| 600–649 | 3 | — | — | 12 | — | — | 1 | — | — | — | — | — | 3 | — | — |
| 650–699 | 13 | — | — | 39 | — | — | 16 | — | — | 4 | — | — | 7 | — | — |
| 700–749 | 37 | — | — | 87 | — | — | 35 | — | — | 6 | — | — | 6 | — | — |
| 750–799 | 53 | — | — | 199 | — | — | 113 | — | — | 46 | — | — | 26 | — | — |
| 800–849 | 64 | — | — | 234 | — | 1 | 214 | — | — | 111 | — | — | 86 | — | — |
| 850–899 | 64 | — | — | 160 | 3 | — | 148 | 1 | — | 155 | — | — | 53 | 1 | 1 |
| 900–949 | 23 | — | — | 66 | 1 | — | 43 | 1 | — | 112 | — | — | 27 | 1 | — |
| 950–999 | 19 | 1 | — | 11 | 3 | — | 16 | 1 | — | 44 | 18 | — | 9 | 3 | — |
| 1000–1049 | 7 | 3 | — | 4 | 5 | — | 10 | 4 | — | 26 | 33 | — | 1 | 3 | — |
| 1050–1099 | 3 | 1 | — | 2 | 4 | — | 7 | 3 | — | 14 | 27 | — | 1 | 1 | — |
| 1100–1149 | — | — | — | 3 | 1 | — | — | 1 | — | 2 | 9 | — | 1 | 2 | — |
| 1150–1199 | — | — | — | — | 3 | — | 1 | 1 | — | — | 3 | — | — | — | — |
| 1200–1249 | — | 1 | — | — | 1 | — | — | 2 | — | — | 1 | — | — | — | — |
| 1250–1299 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| No lengths | | | | 2 | | | 2 | | | 2 | | | 1 | | |
| Total | 287 | 6 | — | 831 | 21 | 1 | 606 | 14 | — | 523 | 91 | — | 221 | 11 | 1 |
| Percent | 97.9 | 2.1 | — | 97.4 | 2.5 | 0.1 | 97.7 | 2.3 | — | 85.1 | 14.9 | — | 94.8 | 4.7 | 0.5 |

508 mm in 1976–82 and 517 mm in 1994–95. All gag >683 mm in the 1976–82 samples and those >750 mm in the 1994–95 samples were sexually mature. During 1994–95, 266 males ($\bar{x}$ TL=1041 mm, SD=68) were collected and no gag less than 875 mm was male (Table 5). During 1976–82, 104 males ($\bar{x}$ TL=1041 mm, SD=73) were collected with the smallest specimen at 795 mm. The transitional specimens collected during 1994–95 were larger than those from 1976 to 1982.

The length at 50% maturity ($L_{50}$) was similar during 1978–82 (641 mm; 95% confidence interval (CI) =616–658 mm) and 1994–95 (622 mm; 95% CI=611–631 mm (Fig. 1); however, the probit analysis with period and total length as independent variables (Pearson $\chi^2$, $P$=0.9953, df=27) indicated that the size at maturity was likely decreasing (Table 6). The negative coefficient for period indicated that overall a smaller proportion of specimens in each size class was mature during 1976–82. The overlap in the histograms of female gag that were developing, ripe, or spent and females that were resting indicated that reproductive tissue was correctly assigned to the immature and resting categories (Fig. 2).

Female gag were in spawning condition from December 1994 through mid May 1995 on the basis of

**Table 5**

Number of mature gag, *Mycteroperca microlepis*, taken during 1994–95 (TL=total length). Trans = transitional specimens.

| Size (mm TL) | Female | Male | Trans |
|---|---|---|---|
| 450–499 | 0 | — | — |
| 500–549 | 2 | — | — |
| 550–599 | 16 | — | — |
| 600–649 | 35 | — | — |
| 650–699 | 121 | — | — |
| 700–749 | 267 | — | — |
| 750–799 | 650 | — | 1 |
| 800–849 | 1021 | — | 2 |
| 850–899 | 790 | 7 | 5 |
| 900–949 | 386 | 14 | 12 |
| 950–999 | 132 | 46 | 10 |
| 1000–1049 | 70 | 82 | 8 |
| 1050–1099 | 33 | 68 | — |
| 1100–1149 | 7 | 28 | — |
| 1150–1199 | 2 | 15 | — |
| 1200–1249 | — | 5 | 1 |
| 1250–1299 | 1 | — | — |
| No Lengths | 41 | 1 | — |
| Total | 3574 | 266 | 39 |
| Percent | 92.1 | 6.9 | 1.0 |

occurrence of hydrated oocytes or postovulatory follicles (Fig. 3). Spawning individuals were caught from North Carolina to Florida by commercial fishermen and fishery-independent sampling (MARMAP). Approximate fishing locations provided by commercial fishermen indicated that gag were spawning from the border between North Carolina and South Carolina to a point east of Ft. Lauderdale, Florida. Spawning individuals were captured on research cruises off South Carolina and Georgia at depths ranging from 49 to 91 m. The spawning season appeared to be more protracted in southern Florida than in other areas, with spawning beginning in December and extending into mid May. Peak spawning occurred from March through mid-April (Fig. 4). When the data were stratified by area, the highest percentage of spawning females was found in northern Florida during March and April (Fig. 4).

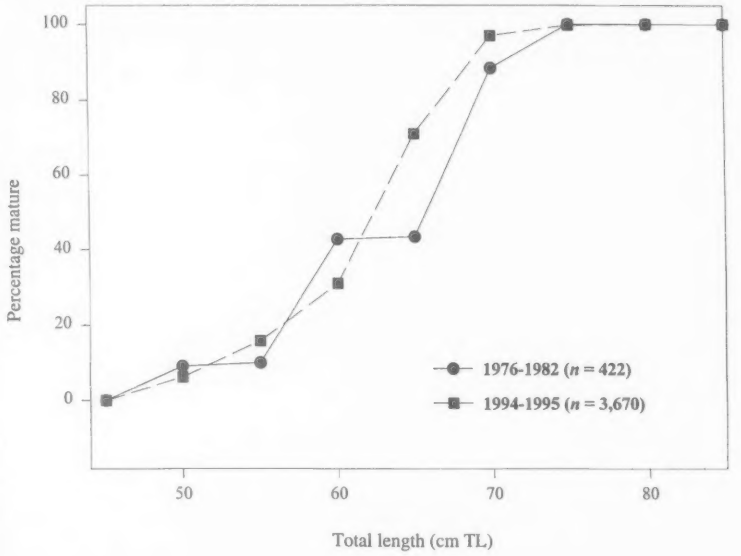


**Figure 1**

Percentage of mature specimens by size class for female gag, *Mycteroperca microlepis*, collected during 1976–82 and 1994–95. Individuals >85 cm are not included in the plot; $n$ = the total number of fish examined.

Overall, spawning of gag declined rapidly after 8 April. Individuals undergoing sexual transition were extremely rare during most of the spawning season; only one transitional specimen was documented during February and March 1994–95, out of 2147 fish examined (Table 7). There was a sharp increase in the number of transitional individuals immediately after the spawning season. The majority of transitional fish ($n$=26) were collected during mid-April through mid-June when 1473 gag gonads were examined. ANOVA and the Duncan multiple range tests indicated that there were significant differences in the mean lengths of gag sampled between months. When the number of males, females, and transitionals between 889 mm and 1050 mm was tabulated

**Table 6**

Results of probit analysis comparing the proportions of mature females in 5-cm length intervals collected during two periods: 1976–82 and 1994–95 (TL=total length).

| Parameter | Estimate | SE | P |
|---|---|---|---|
| Intercept | −12.304 | 0.871 | <0.0001** |
| Period | −0.378 | 0.176 | 0.0320* |
| cm TL | 0.192 | 0.013 | <0.0001** |

* = significant at $P$<0.05; ** = significant at $P$<0.0001.

**Table 7**

Total number sampled ($n$), mean total length (mm TL; sexes combined), standard deviation (SD), number of males (M), number of females (F), and number of transitional specimens (T) by month for mature gag captured during 1994 and 1995. Means with same letter are not significantly different ($P$>0.05).

| Month | $n$ | $\bar{x}$ TL | SD | M | F | T |
|---|---|---|---|---|---|---|
| Jan | 243 | 837.0$^{bcd}$ | 85.5 | 10 | 229 | 4 |
| Feb | 754 | 829.7$^{bcd}$ | 87.0 | 27 | 726 | 1 |
| Mar | 1142 | 859.0$^{b}$ | 99.2 | 87 | 1055 | — |
| Apr | 1027 | 844.8$^{bc}$ | 86.4 | 50 | 965 | 12 |
| May | 297 | 873.2$^{ab}$ | 110.8 | 48 | 235 | 14 |
| Jun | 48 | 928.1$^{a}$ | 110.7 | 11 | 32 | 5 |
| Jul | 77 | 925.9$^{a}$ | 124.1 | 19 | 56 | 2 |
| Aug | 2 | 717.0$^{f}$ | 106.1 | — | 2 | — |
| Sep | 33 | 774.8$^{ef}$ | 84.1 | 2 | 31 | — |
| Oct | 24 | 759.7$^{ef}$ | 83.6 | — | 24 | — |
| Nov | 77 | 818.5$^{de}$ | 86.8 | — | 76 | — |
| Dec | 89 | 788.5$^{cde}$ | 89.7 | 2 | 86 | 1 |

to reduce the potential for bias that might result from the selection of larger fishes after 18 April 1995, the Duncan multiple range test showed little difference in the mean size of gag collected each month (Table 8), and it was still apparent that transitional fish did not appear in the collections until immediately after the spawning season.

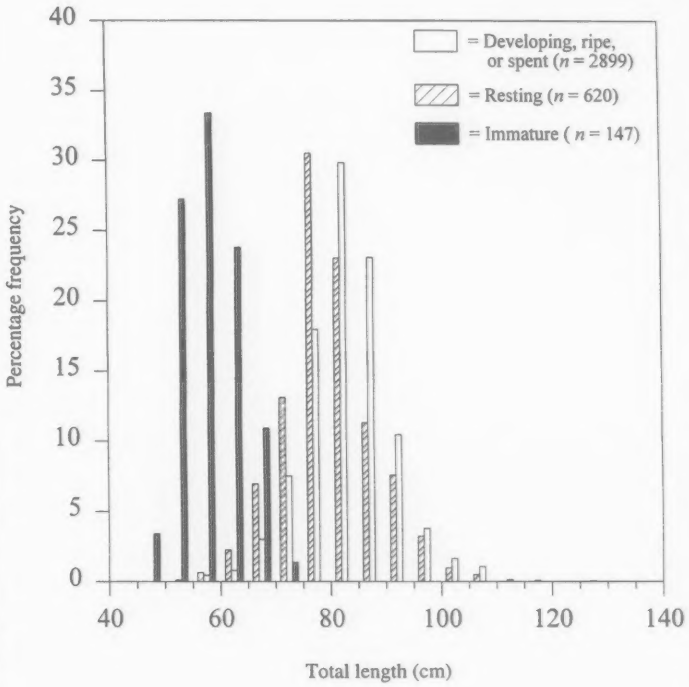


**Figure 2**

Comparison of the size frequency of immature gag, *Mycteroperca microlepis*, with the size frequencies of resting females and females with evidence of certain maturity.

## Discussion

Analysis of reproductive data indicates that the gag population along the southeast coast of the United States is stressed. The percentage of males in the adult population has decreased from 19.6% (1976–82) to 5.5% (1994–95). However, the percentage of males currently in the population may actually be less than 5.5%; more recent data were collected during the spawning season when male gag are most vulnerable to fishing gear (Coleman et al., 1996). In contrast, data were collected throughout the year during 1976–82 rather than primarily during the spawning season. Had the 1995 emergency rule been put into place at a time of the year when gag were not spawning, it is possible that fewer males would have been captured.

The relative abundance of males and the length of the spawning period were greater off Florida than off other southeastern states. The highest percentage of males (14.9%) during 1995 was noted off northern Florida. However, the majority of males were taken by one fisherman on 2 March 1995, 17 March 1995, and 1 April 1995 indicating that this fisherman may have been targeting a relatively unfished spawning aggregation. Spawning aggregations of gag (Coleman et al., 1996) and red hind, *Epinephelus guttatus* (Shapiro et al., 1993a), contained a higher percentage of males than nonaggregation groups. Commercial fishermen indicate that after the spawning season (May and June), female gag move in groups to shallower water (~30 m) and the larger males become solitary and remain at depths of 50 to 90 m.

Probit analysis revealed that female gag may have been maturing at smaller sizes during 1994–95 than during 1976–82 which would further indicate that

**Table 8**

Total number sampled ($n$), mean total length (mm TL; sexes combined), standard deviation (SD), number of males (M), number of females (F), and number of transitional specimens (T) by month for mature gag (>889 mm and <1050 mm) captured during 1994 and 1995. Means with same letter are not significantly different ($P$>0.05).

| Month | $n$ | $\bar{x}$ TL | SD | M | F | T |
|---|---|---|---|---|---|---|
| Jan | 45 | $932.4^{b}$ | 41.5 | 5 | 38 | 2 |
| Feb | 126 | $935.6^{b}$ | 42.5 | 12 | 114 | — |
| Mar | 308 | $948.3^{ab}$ | 46.4 | 51 | 257 | — |
| Apr | 219 | $939.6^{ab}$ | 43.8 | 27 | 183 | 9 |
| May | 92 | $949.8^{ab}$ | 43.4 | 24 | 54 | 14 |
| Jun | 25 | $971.0^{ab}$ | 49.7 | 7 | 13 | 5 |
| Jul | 41 | $963.1^{ab}$ | 46.7 | 10 | 30 | 1 |
| Aug | 0 | — | — | — | — | — |
| Sep | 3 | $986.7^{a}$ | 28.9 | 2 | 1 | — |
| Oct | 2 | $944.5^{ab}$ | 77.1 | — | 2 | — |
| Nov | 10 | $961.6^{ab}$ | 52.7 | 1 | 9 | — |
| Dec | 11 | $933.0^{b}$ | 47.4 | 2 | 8 | 1 |

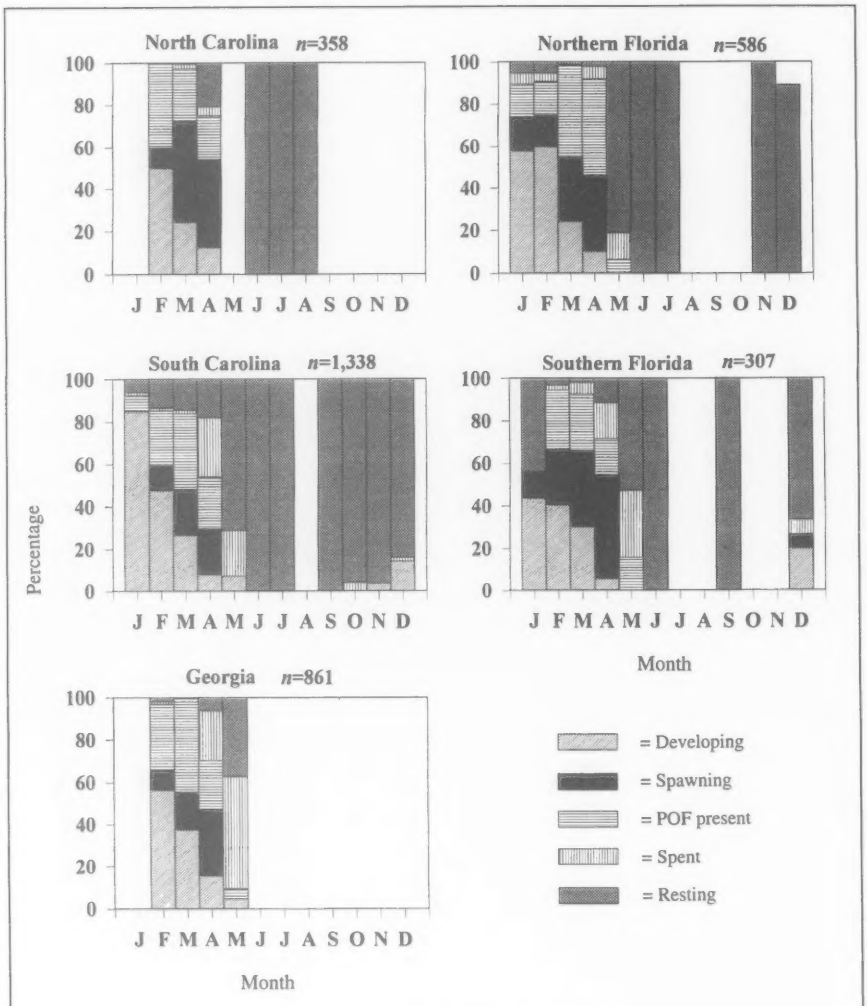


**Figure 3**

Reproductive seasonality of female gag, *Mycteroperca microlepis*, in North Carolina, South Carolina, Georgia, northern Florida, and southern Florida during 1994–95. POF = postovulatory follicles.

the gag population is stressed. Although probit analysis suggested size at maturity is decreasing, it is difficult to conclude that this is happening in the population on the basis of estimates of $L_{50}$ because there is overlap in the confidence intervals. A cautious interpretation is also warranted given the small number of females ($n$=422) sampled during 1976–82. Coleman et al. (1996) found that female gag collected in the Gulf of Mexico during 1991–93 became sexually mature and underwent transition at smaller sizes than those reported by Hood and Schlieder (1992) for the same region during 1977–80. Changes in life history aspects of gag from the Gulf of Mexico were attributed to steadily increasing fishing pressure.

Gag were in spawning condition from December through mid May in southern Florida, January through May in northern Florida, and January through April in South Carolina. Although no specimens were collected during January in North Carolina and Georgia, individuals in spawning condition were landed during February through April. Peak spawning activity occurred from March through mid-April in all areas. Collins et al. (1987) and Keener et al. (1988) also reported that peak spawning occurred

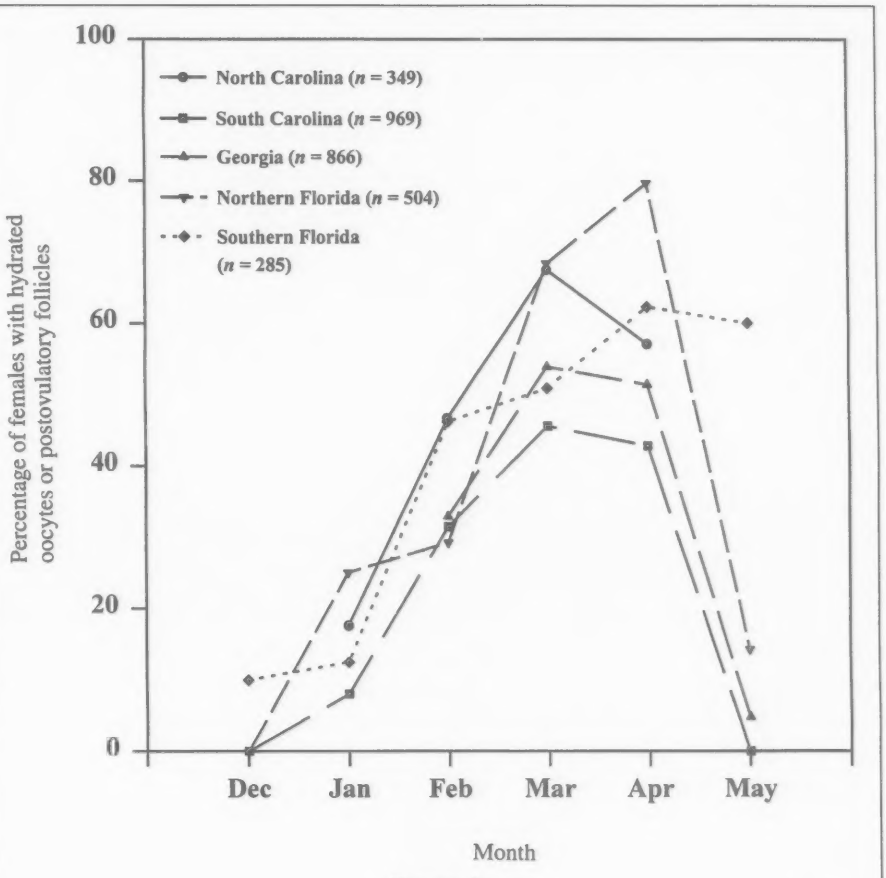


**Figure 4**

Percentage of female gag, *Mycteroperca microlepis*, in spawning condition by area during 1994–95.

in March and April along the southeast Atlantic coast. Hood and Schlieder (1992) and Coleman et al. (1996) indicated that peak spawning occurred during February and March in the Gulf of Mexico.

Despite the large number of specimens examined during 1994–95 ($n$=3879), transitional specimens ($n$=39) made up a small percentage (1.0%) of individuals collected. The low number of individuals with transitional gonads may have been due to the rapid nature of sex transition that appears to occur immediately after the spawning season. Smith (1965) and Moe (1969) suggested that other groupers may have a quick rate of sex transition. Because there was no significant monthly difference in the mean length of gag between 889 and 1050 mm TL landed during January through July, we feel that the peak in the number of transitional specimens during April and May was real and not an artifact of the emergency rule that required fishermen to land only larger fish (>889 mm TL) after 18 April. Coleman et al. (1996) stated that sex transition in gag and other grouper species may be socially mediated either through size ratio or sex ratio cues. Shapiro et al. (1993b) proposed that one function of annual spawning aggregations in red hind may be that of enabling a female to determine if it should function as a female or a male during the next spawning season. Shapiro et al. (1993b) further suggested that if this hypothesis is true, sex-changing individuals should be found soon after the aggregation has dispersed. The high percentage of males and transitionals taken during April and May in this study indicates that aggregations may remain intact for a short time after the spawning season.

Van Sant et al. (1994) and MARMAP tagging data[1] provided evidence that some gag migrate from off-

[1] 1998. Unpublished data from MARMAP tagging study. Marine Resources Research Institute, Charleston, SC.

shore areas of South Carolina and Georgia to the shelf edge off Florida, perhaps to spawn. However, the presence of hydrated oocytes and new postovulatory follicles in female gag taken from North Carolina through Florida, including specific locations off South Carolina and Georgia, shows that spawning is not restricted to Florida. Hunter et al. (1985) reported that the presence of hydrated oocytes in teleosts is an indication that individuals will spawn within 12 hours. Furthermore, Hunter et al. (1986) found that postovulatory follicles are present only in fishes that have recently spawned.

Gag off Florida are subject to intense fishing pressure by commercial fishermen, sport divers, and anglers, particularly during the late fall through early spring, because the continental shelf is very narrow and spawning aggregations are easily accessed. Therefore, even if most gag are not migrating to Florida for spawning, the vulnerability of at least part of the stock to winter fishing is of concern. This "funneling" of migratory fishes off southeastern Florida may increase fishing mortality for many species and requires further investigation.

In 1993, the SAFMC decided to take no action on a proposal to close all fishing for gag during their spawning season because insufficient data were available on sex ratios and the spatial extent of spawning along the southeast coast of the United States. The information on sex ratio and size at maturity presented here indicates that the gag population is overfished. Because there has been a reduction in the percentage of males in commercial landings and a modest decrease in the size at maturity for females, the total fishing effort should be limited, especially in the removal of males from spawning aggregations. Since gag spawn in offshore areas from North Carolina to southeast Florida, any regulation should apply to all southeastern states. However, seasonal closure to fishing may not be sufficient to protect gag from overexploitation because males will continue to be lost during the open season. Protected areas with no fishing, such as marine fishery reserves, may be a possible solution to overfishing of reef fishes along the southeast coast of the United States (PDT, 1990). Marine fishery reserves can protect population age structure, species diversity, genetic diversity, and recruitment supply to exploited areas (Bohnsack, 1993). Sedberry et al. (1996) found that marine reserves in Belize, Central America, had a higher diversity of fishes than in areas that were not protected. Top predators, such as various grouper and snapper species, were more abundant and larger in reserves. In addition, populations of herbivorous forage species were reduced to presumed natural levels in the presence of protected predators. Marine reserves in Belize appear to have a natural balance of predators and forage species in relation to fished areas. There are other species in the snapper-grouper complex that are showing signs of overfishing, in addition to gag, including red porgy (Harris and McGovern, 1997), vermilion snapper (Zhao and McGovern, 1997; Zhao et al., 1997), and black sea bass.[2] In addition, less important species in fisheries (e.g. white grunt, gray triggerfish) are increasing in abundance.[2] This trend is likely to continue unless different management regulations are imposed that will protect ecosystems and restore a natural equilibrium community.

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## Literature cited

**Bohnsack, J. A.**
**1993.** Marine reserves. Oceanus 36:63–71.

**Carter, J., G. J. Marrow, and V. Pryor.**
**1994.** Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proc. Gulf Caribb. Fish. Inst. 43:65–111.

**Coleman, F. C., C. C. Koenig, L. A. Collins.**
**1996.** Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fish. 47:129–141.

[2] 1998. MARMAP unpublished data. Marine Resources Research Institute, South Carolina Department of Natural Resources, Charleston, SC 29422-2559.

**Collins, M. R., and G. R. Sedberry.**
**1991.** Status of vermilion snapper and red porgy stocks off South Carolina. Trans. Am. Fish. Soc. 120:116–120.

**Collins, M. R., C. A. Waltz, W. A. Roumillat, and D. L. Stubbs.**
**1987.** Contribution to the life history and reproductive biology of gag, *Mycteroperca microlepis* (Serranidae), in the South Atlantic Bight. Fish. Bull. 85:648–653

**Cuellar, N., G. R. Sedberry, and D. M. Wyanski.**
**1996.** Reproductive seasonality, maturation, fecundity, and spawning frequency of the vermilion snapper, *Rhomboplites aurorubens*, off the southeastern United States. Fish. Bull. 94:635–653.

**Ferreira, B. P.**
**1993.** Reproduction of the inshore coral trout *Plectropomus maculatus* (Perciformes: Serranidae) from the Central Great Barrier Reef, Australia. J. Fish. Biol. 42:831–834.

**Gilmore, R. G., and R. S. Jones.**
**1992.** Color variation and associated behavior in the epinepheline groupers, *Mycteroperca microlepis*, (Goode and Bean) and *M. phenax* Jordan and Swain. Bull. Mar. Sci. 51:83–103.

**Hardy, J. E., Jr.**
**1978.** Development of fishes of the Mid-Atlantic Bight—an atlas of egg, larval, and juvenile stages, vol. III: Aphredoderidae through Rachycentridae. U.S. Fish and Widl. Serv., U.S. Dep. Interior, 394 p.

**Harris, P. J. and J. C. McGovern.**
**1997.** Changes in the life history of red porgy, *Pagrus pagrus*, from the southeastern United States, 1972–1994. Fish. Bull. 95:732–747.

**Hood, P. B., and R. A. Schlieder.**
**1992.** Age, growth, and reproduction of gag *Mycteroperca microlepis*, (Pisces: Serranidae), in the eastern Gulf of Mexico. Bull. Mar. Sci. 51:337–352.

**Hunter, J. R., N. C. H. Lo, and R. J. H. Leong.**
**1985.** Batch fecundity in multiple spawning fishes. *In* R. Lasker (ed.), An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*), p. 67–77. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.

**Hunter, J. R., and B. J. Macewicz.**
**1985.** Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fish. Bull. 83:119–136.

**Hunter, J. R., B. J. Macewicz, and J. R. Sibert.**
**1986.** The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the South Pacific. Fish. Bull. 84:895–903.

**Huntsman, G. R.**
**1976.** Offshore headboat fishing in North Carolina and South Carolina. Mar. Fish. Rev. 38(3):13–23.

**Keener, P., G. D. Johnson, B. W. Stender, E. B. Brothers and H. R. Beatty.**
**1988.** Ingress of postlarval gag, *Mycteroperca microlepis* (Pisces: Serranidae), through a South Carolina barrier island inlet. Bull. Mar. Sci. 42:376–396.

**Moe, M. A., Jr.**
**1969.** Biology of the red grouper, *Epinephelus morio* (Valenciennes), from the eastern Gulf of Mexico. Fla. Dep. Nat. Resour. Mar. Res. Lab., Prof. Pap. 10, 95 p.

**Nelson, K., and M. Soulé.**
**1987.** Genetic conservation of exploited fishes. *In* N. Ryman and F. Utter (eds.), Population genetics and fishery management, p. 345–368. Univ. Washington Press, Seattle, WA.

**PDT (Snapper-Grouper Plan Development Team).**
**1990.** The potential of marine fishery reserves for reef fish management in the U.S. South Atlantic. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-SEFC-216, 45 p.

**SAFMC (South Atlantic Fishery Management Council).**
**1993.** Amendment number 6, regulatory impact review and final environmental impact statement for the snapper grouper fishery of the South Atlantic region. SAFMC, Charleston, SC, 189 p.

**SAS Institute, Inc.**
**1990.** SAS/STAT® user's guide, version 6, 4th edition, vol. 2. SAS Institute, Inc, Cary, North Carolina, p. 1325–1350.

**Sedberry, G. R., J. Carter and P. A. Barrick.**
**1996.** A comparison of fish communities between protected and non-protected areas of the Belize Barrier Reef ecosystem: Implications for conservation and management. Proc. Gulf Caribb. Fish. Inst. 45:95–127.

**Shapiro, D. Y., Y. Sadovy, and M. A. McGehee.**
**1993a.** Periodicity of sex change and reproduction in the red hind, *Epinephelus guttatus*, a protogynous grouper. Bull. Mar. Sci. 53:1151–1162.
**1993b.** Size, composition, and spatial structure of the annual spawning aggregation of the red hind, *Epinephelus guttatus* (Pisces: Serranidae). Copeia 1993:399–406.

**Smith, C. L.**
**1965.** The patterns of sexuality and the classification of serranid fishes. Am. Mus. Novit. 2207:1–20.
**1971.** A revision of American groupers: *Epinephelus* and allied genera. Bull. Am. Mus. Nat. Hist. 146:67–242.

**Smith, P. J., R. I. C. C. Francis, and M. McVeagh.**
**1991.** Loss of genetic variation due to fishing pressure. Fish. Res. 10:309–316.

**West, G.**
**1990.** Methods of assessing ovarian development in fishes: a review. Aust. J. Mar. Freshwater Res. 41:199–222.

**Van Sant, S. B., M. R. Collins, and G. R. Sedberry.**
**1994.** Preliminary evidence from a tagging study for a gag (*Mycteroperca microlepis*) spawning migration, with notes on the use of oxytetracycline for chemical tagging. Proc. Gulf Caribb. Fish. Inst. 43:417–428.

**Zhao, B., and J. C. McGovern.**
**1997.** Temporal variation in sexual maturity and gear-specific sex ratio of the vermilion snapper in the South Atlantic Bight. Fish. Bull. 95:837–848.

**Zhao, B., J. C. McGovern, and P. J. Harris.**
**1997.** Age, growth, and temporal change in size at age of the vermilion snapper from the South Atlantic Bight. Trans. Am. Fish. Soc. 126:181–193.

# Distribution and abundance of and habitat use by harbor porpoise, *Phocoena phocoena*, off the northern San Juan Islands, Washington

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**Abstract.**—Boat surveys along randomly placed line transects were conducted from June to August 1991 and June to October 1992 to determine distribution and abundance of and habitat use by harbor porpoise (*Phocoena phocoena*) off the northern San Juan Islands, Washington. There were 301 sightings (average 4.4 sightings/h) of 526 harbor porpoise during 73 random boat surveys, with group sizes of 1 to 8 individuals (mean=1.87, SE=0.06, $n$=278). An estimated 299 harbor porpoise (1.26 porpoise/km$^2$, SE=0.20) were distributed in an aggregated pattern within a 237 km$^2$ area (10% of Washington Sound), indicating that a large proportion (30%) of harbor porpoise in Washington Sound occur in the northern San Juan Islands. Harbor porpoise were distributed over a depth range from 20.1 to 235.0 m (mean= 141.6 m, SE=2.43, $n$=275) and were observed more than expected ($P$<0.05) in depths greater than 125 m and over shallow slopes (<10%) and observed less than expected ($P$<0.05) in depths less than 75 m. Porpoise occurred at sea surface temperatures of 10.1° to 16.3°C and were sighted more frequently than expected ($P$<0.05) in water temperatures of 11° to 12°C. Boat surveys along fixed location transects indicated distribution was similar between 1991 and 1992. The occurrence of harbor porpoise in deep water, at cooler sea surface temperatures, over shallow sloping seafloor, and in tidally mixed regions (owing to currents and tide rips) within our study area may, collectively, affect prey distribution and associated harbor porpoise distribution.

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Harbor porpoise (*Phocoena phocoena*) are present year-round off the coast and inland waters of Washington State. Historically, harbor porpoise have been present throughout the Strait of Juan de Fuca, Washington Sound (San Juan Island archipelago), and south in Puget Sound. Once considered the most common cetacean in southern Puget Sound (Scheffer and Slipp, 1948), harbor porpoise sightings are now rare (Everitt et al., 1980; Calambokidis et al., 1984, 1985). Although harbor porpoise have not been sighted off the central San Juan Islands in recent years (Flaherty and Stark[1]; Calambokidis et al.[2]), sightings off the northern San Juan Islands have been common (Flaherty and Stark[1]; Calambokidis et al.[2]). Reasons for the disappearance of harbor porpoise from South Puget Sound are unclear but may be due to reduced availability of prey (because of changes in environmental conditions), fishing pressure, disturbance, net entanglement, or pollution.

Many biological (e.g. prey) and physical oceanographic factors (e.g. depth, seafloor relief, tidal currents, and sea surface temperature) affect the distribution of cetaceans. Increased availability of prey in deep waters may be a factor affecting the distribution of harbor porpoise. Smith and Gaskin (1983) found a significant positive correlation between abundance of mother-and-calf pairs and bottom depth and copepod (*Calanus* spp.) density. Abundance of harbor porpoise also was positively correlated with depth and physiographic features that concentrated Atlantic herring (*Clupea harengus*) in near-surface waters (Watts and Gaskin, 1985). In Fish Harbor, New Brunswick, Canada, harbor porpoise were associated with reduced sea surface temperatures that coincided with a large influx of juvenile herring in the region (Gaskin and Watson, 1985). Tidal state affected movements of harbor porpoise in the Bay of Fundy; har-

[1] Flaherty, C., and S. Stark. 1982. Harbor porpoise (*Phocoena phocoena*) assessment in "Washington Sound." Final report for subcontract 80-ABA-3584. National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA, 84 p.

[2] Calambokidis, J., J. C. Cubbage, J. R. Evenson, S. J. Jeffries, and R. F. Brown. 1993. Abundance estimates of harbor porpoise in Washington and Oregon waters. Final Report to National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA, 55 p.

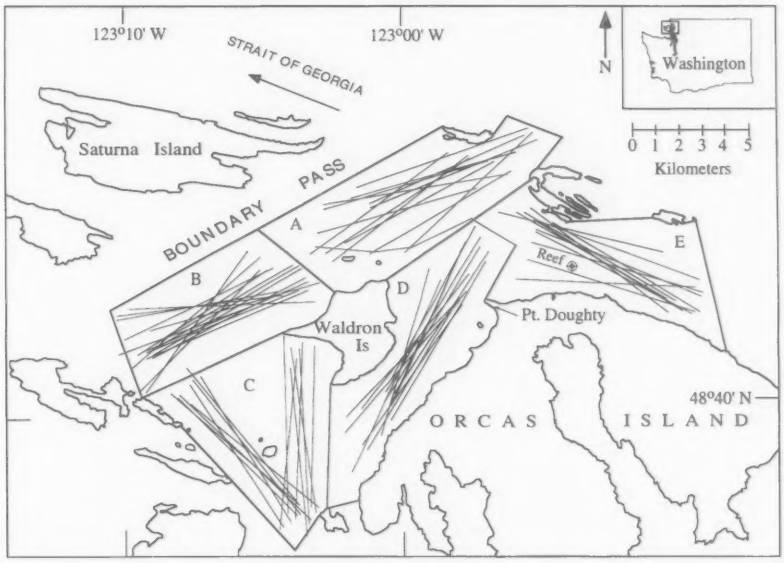


**Figure 1**

Random boat transects ($n$=73) completed from June to October 1992 within five stratified sections (A, B, C, D, E), northern San Juan Islands, Washington.

bor porpoise were observed more often during flood tide than ebb tide (Watson, 1976) and moved inshore during flood tides and offshore during ebb tides (Gaskin and Watson, 1985).

Harbor porpoise in northern Puget Sound are vulnerable to some of the same detrimental effects (disturbance, net entanglement, and pollution) that may have caused the disappearance of harbor porpoise in southern Puget Sound. It is important, therefore, to determine the abundance of harbor porpoise and identify habitat variables that may influence their distribution in northern Puget Sound. The main objectives of this study were to determine 1) the spatial and temporal distribution, density, and abundance of harbor porpoise occurring off the northern San Juan Islands and 2) the relation of harbor porpoise to depth and percentage slope of the seafloor, sea surface temperature (SST), tidal state, and time of day.

## Methods

### Study area

Washington Sound is located in the northwest corner of Washington State (48°15' to 48°50'N and 122°27' to 123°13'W), between the southern portion of Vancouver Island and the mainland, from Fidalgo Island to north of Vancouver, including the American and Canadian islands of the San Juan Archipelago (Kozloff, 1973). Mean diurnal tide heights are between 1.3 and 2.9 m (NOAA, 1991). Northern Washington Sound (northern San Juan Islands) has numerous islands and reefs with deep channels, strong currents, and tide rips. The study area off the northern San Juan Islands (Fig. 1) was selected on the basis of preliminary boat surveys conducted in 1991 to determine areas of harbor porpoise occurrence. Additionally, information was obtained from local residents and The Whale Museum, Friday Harbor, Washington.

### Random boat surveys

Randomly located boat transects ($n$=73; Fig. 1) were conducted from 27 June to 2 October 1992 within a study area composed of five strata (lettered A–E; Fig. 1) to determine harbor porpoise distribution, density, abundance, and habitat use. Eight-km transects were located within each approximately equal (42 to 50 km$^2$) stratum by using random starting points and random compass bearings. Strata were originally chosen so that transects would adequately cover the entire study area. Because placement of straight 8-km transects was constrained by the boundaries of strata and islands or reefs within strata, however, some regions of each strata were not adequately sampled. When sea conditions permitted, the five

strata, hereafter referred to as sections, were surveyed on the same day. Sections were chosen in a random starting order and every attempt was made to complete all sections before surveying the same sections again.

Harbor porpoise were surveyed from a 7.3-m aluminum marine patrol vessel during Beaufort sea state 0 (wind speed=0–1.8 km/h), 1 (wind speed=1.8–5.6 km/h), or rarely Beaufort 2 (wind speed=7.4–11 km/h). Each transect was completed in approximately 52 min, at an average boat speed of 9 km/h. Date, time, and tidal phase (flood or ebb) were recorded before each transect was surveyed. At the beginning and end of each transect, Secchi disk readings were recorded to the nearest 0.1 m and SST was recorded (by a calibrated thermometer located on the transducer of the survey vessel) to the nearest 0.1°C. During surveys, two observers divided the field of view across the forward 180° of the transect path. Observations were made from the roof of the vessel (height above the waterline=2.68 m, measured to the observers' eyes in surveying position) with unaided eyes and with Fujinon 7 × 50 reticle and compass binoculars. When an individual or group of harbor porpoise was located, an observer recorded time; group size and composition; compass bearing to the porpoise; ocular reticle marks from the horizon to the porpoise; Beaufort sea state; number of boats, birds, and marine mammals within 1 km of vessel; and direction of harbor porpoise travel. A group of harbor porpoise was defined as two or more porpoise visible at the water's surface within three body lengths (5 m) of each other, having nearly synchronous diving patterns (<15 seconds between sightings of each individual). Observers were trained and tested in the use of reticle binocompasses and in calibrating their readings on buoys and points of land and comparing distance accuracy to National Oceanic and Atmospheric Administration (NOAA) navigational charts and vessel's radar. Compasses on binoculars were also tested and were found not to be significantly affected by metal on the survey vessel. Although not common, the horizon was sometimes obscured by land in the observers' viewing area. To compensate for this, we estimated the number of reticles from the land-water interface (directly beyond the porpoise) to the porpoise sighting, then carefully rotated the binoculars from the land to the horizon. We then determined the number of reticles the horizon was beyond the land and added this amount to the reticle reading of the harbor porpoise sighting. Loran coordinates, Beaufort sea state, visibility, SST, and number of boats within 1 km of the vessel were recorded every ten minutes during surveys and for each harbor porpoise sighting. Depth and seafloor slope (at each harbor porpoise sighting) were determined from NOAA navigational charts and bathymetric charts.

Locations of harbor porpoise were determined with the aid of Fujinon 7 × 50 reticle (one reticle=17 min or 0.283°) and compass binoculars. Vertical angle was calculated as the angle between the horizon and the harbor porpoise. Distance to harbor porpoise was calculated as

$$D_r = \frac{H}{\tan(\alpha)},$$

where $D_r$ = the radial distance from the vessel to the porpoise;
$H$ = the eyeheight of observers; and
$\alpha$ = the vertical angle between the horizon and porpoise.

Locations were plotted on NOAA navigational charts by using Loran (latitude and longitude) coordinates of the vessel at the time harbor porpoise were sighted, and distance and bearing to the sighting.

Perpendicular distance from the trackline to harbor porpoise was determined by using

$$D_p = D_r \times \sin(\alpha),$$

where $D_p$ = perpendicular distance;
$D_r$ = the radial distance to the harbor porpoise; and
$\alpha$ = the angle off the trackline (the difference between the trackline heading and the bearing to porpoise).

Seafloor depth and slope were determined by using a NOAA navigational chart and bathymetric map. Percentage slope was calculated as

$$\%\, slope = \frac{dz}{ds} \times 100\%,$$

where $dz$ = the difference between the two closest depths (m) printed on the chart on either side of a harbor porpoise location (with contour lines drawn among depths); and
$ds$ = the distance (m) between those two depths.

Bathymetric charts with contour intervals of 10 m were used to verify angle of slope between depths.

To determine if harbor porpoise occurred over depths and slopes in proportion to available depths

and slopes in the study area, eight random points were plotted within a 2-km strip along the length of each 8-km transect ($n$=73 transects, 584 points) and depth and slope were determined for each point. The number of random points chosen was determined by plotting precision (standard deviation/mean) against sample size until there was little variability in this measure (i.e. a plateau and subsequent leveling of the curve).

Density and abundance estimates were calculated by using the line transect method as described by Burnham et al. (1980) and the computer program DISTANCE (Laake et al., 1993). Each transect was considered a replicate. Density and variance estimates of harbor porpoise sightings ($n$=250) were calculated by replicate for each section ($n$=12 to 15 transects) and by replicate for all sections combined ($n$=70 transects). Transects with Beaufort sea state of 2 ($n$=3) were deleted from analyses because sighting rates of harbor porpoise in Beaufort 2 are less than Beaufort 0 or 1 (Barlow, 1988). Density was calculated as

$$D = \frac{n \times f(0) \times s}{2L},$$

where $n$ = number of individual harbor porpoise sightings;
$f(0)$ = the probability density function of distances from the trackline evaluated at zero distance;
$s$ = average group size of harbor porpoise sightings, and
$L$ = total length of the trackline.

Abundance was calculated as density multiplied by area of each section (A–E) and all sections (237 km$^2$). The parameter $f(0)$ is essentially a measure of sighting efficiency and should not vary with porpoise abundance as long as sighting conditions (e.g. Beaufort sea state, visibility) remain the same. Because we surveyed only during optimal sighting conditions (Beaufort ≤1, no rain or fog) within all sections and because relatively large sample sizes are required to estimate $f(0)$ accurately, values of $f(0)$ for each section were estimated by pooling all sightings in all sections. Effective strip width is defined as $1/f(0)$, which equals one-half the transect width, such that as many objects are detected outside the strip as remain undetected within it (Buckland et al., 1993). Because group size was independent of distance from the trackline (determined through size-bias regression analysis with DISTANCE software), average group size was used to calculate density. Average group size was estimated by section and for all sightings combined.

Uniform, half-normal (hermite), hazard rate, and negative exponential models were compared with the frequency distributions of perpendicular sighting distance of harbor porpoise to trackline with DISTANCE. Several groupings and truncation points were investigated to achieve the best model fit. Buckland et al. (1993) recommend truncating 5 to 10% of objects detected at the greatest distances from the trackline. The half-normal (hermite) model, grouped into 50 m intervals and truncated at 750 m (deleting 5% of sightings), was chosen on the basis of lowest Akaike Information Criterion (AIC; Buckland et al., 1993) score for all sections combined.

The probability of detection at zero perpendicular distance, $g(0)$, was assumed to be one (all harbor porpoise on the trackline were assumed to be seen) because we were unable to estimate perception bias (bias resulting from animals available to be seen but that were not; Marsh and Sinclair, 1989). We did not have an independent observer to watch the trackline for porpoise that were missed by our two observers, therefore, a correction was not applied to $g(0)$. It is likely $g(0)$ was less than one but it is probably high (slow boat speed and excellent sighting conditions). Because $g(0)$ was constant over the survey time period, the habitat correlations are valid; however, the abundance estimate is underestimated by an unknown amount.

Seafloor depth and slope available in the study area in relation to areas of harbor porpoise occurrence were compared by using chi-square goodness-of-fit analyses. To test whether the frequency of occurrence of harbor porpoise was independent of frequency of tidal currents and surface temperature, we also used chi-square goodness-of-fit analyses. More surveys were conducted during flood tide ($n$=52) than during ebb tide ($n$=17); therefore, the number of harbor porpoise observed per minute during flood or ebb tide was used to standardize the data. A Mann-Whitney $U$, nonparametric two-sample test was conducted to examine differences in number of harbor porpoise observed per minute for each transect ($n$=73) during flood and ebb tides.

Power analyses (Cohen, 1988) were conducted on nonsignificant categories of chi-square goodness-of-fit analyses. Randomization statistics with the program Resampling Stats (Resampling Stats, 1995) were performed to assess the probability of detecting a difference between flood and ebb tides when the difference was determined to be nonsignificant. Additionally, power analyses were used to estimate the probability of detecting trends in abundance over time (Gerrodette, 1987).

Not all times of day were sampled equally; therefore, abundance of harbor porpoise in relation to time of day was compared by using number of porpoise observed per minute to standardize the data. Mean number of harbor porpoise observed per minute for each hour of daylight was compared with Kruskal-Wallis nonparametric analysis of variance and Kolmogorov-Smirnov goodness-of-fit analyses (Zar, 1984). Nonparametric statistics were used for data with non-normal distributions or unequal variances.

### Fixed boat surveys

To determine temporal changes in harbor porpoise distribution between 1991 and 1992, six 8-km transect lines (hereafter called fixed transects; Fig. 2), placed in areas of harbor porpoise occurrence (preliminary harbor porpoise surveys and information from Orca Hotline, The Whale Museum, Friday Harbor, Washington), were surveyed regularly from 27 July to 26 August 1991 and from 24 July to 28 August 1992. In 1991, there was only one observer per survey; therefore, only one half the transect (bow out to 90° port or starboard) was completed during each survey. To be consistent in 1992, one observer surveyed from bow to 90° port while the other surveyed from bow to 90° starboard so that one half of each transect could randomly be compared to 1991 transects.

Harbor porpoise were counted from the same 7.3-m vessel as in random surveys during a Beaufort sea state of 0 or 1. Each fixed transect survey was conducted at an average speed of 11 km/h and completed in 40 to 45 minutes. This vessel speed was chosen in 1991, and to be consistent, 1992 fixed transect surveys were conducted at the same speed (instead of 9 km/h as in random boat surveys). Harbor porpoise locations were calculated as in random boat survey methods.

Mean number of sightings of harbor porpoise per survey between 1991 and 1992 was compared by using a $t$-test. Because both sides of the vessel were observed during a single survey in 1992, each side could not be considered an independent sample. Therefore, one side of the vessel was randomly chosen from each survey in 1992 to compare with 1991. Power tests (Cohen, 1988) were performed when results were not significant.

## Results

### Random boat surveys

There were 301 sightings of 526 harbor porpoise (Fig. 3) during random boat surveys. Of these, 20 sightings (39 porpoise) were possible resightings (i.e. observer believed the porpoise had already been seen during that survey, given the location and direction of travel of porpoise), therefore, these possible resightings were not used in analyses. An average of 4.4 harbor porpoise sightings were recorded per hour (8.1 harbor porpoise per hour), with group sizes of 1 to 8 (mean=1.87, SE=0.06, $n$=278) individuals. Thirteen cow and calf pairs were observed between June and September. Harbor porpoise were sighted during 75% of surveys at a mean perpendicular distance of 237 m (SE=13.89, $n$=250, range: 0 to 1060 m) from the trackline. The half-normal (hermite) model, truncated at 750 m, best fitted the frequency distribution of perpendicular distance of harbor porpoise sighted from the trackline (Fig. 4). Using harbor porpoise sightings ($n$=250) for all sec-

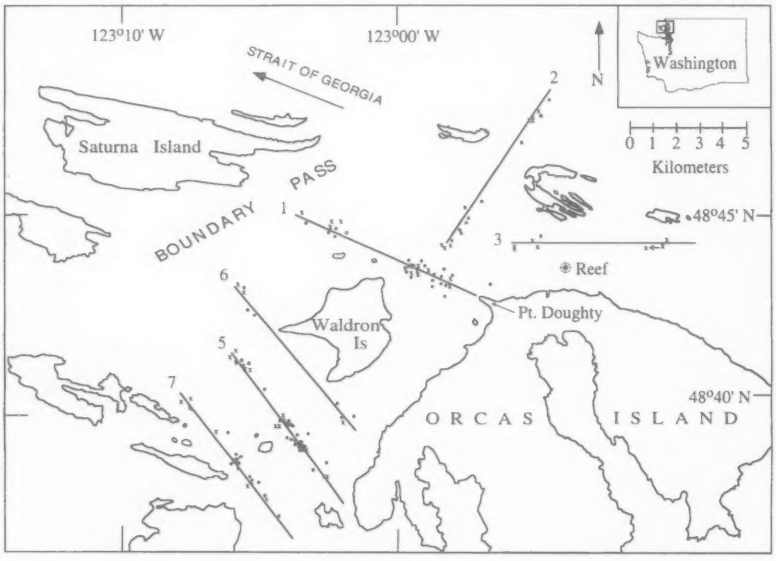


**Figure 2**

Harbor porpoise locations along fixed boat transects (1, 2, 3, 5, 6, 7) in 1991 and 1992 off the northern San Juan Islands, Washington. The "x" denotes locations of harbor porpoise sighted in 1991; the "•" denotes locations in 1992.

tions combined, we estimated that the effective half-strip width (ESW) was 337 m (95% CI=307–371 m; coefficient of variation, CV=0.048), with an $f(0)$ of 2.96/km (SE=0.14, CI=2.70–3.25, CV=0.048).

No significant correlation ($r$=0.097, $n$=250, $P$=0.938) was detected between harbor porpoise group size and perpendicular distance from the trackline. For surveys conducted during Beaufort ≤1 ($n$=70) in all sections (A–E), the mean group size was 1.91 harbor porpoise (SE=0.07, $n$=250; Table 1) and mean density was 1.26 harbor porpoise/km$^2$ (SE=0.20; Table 1). Harbor porpoise densities were least in section A (0.60 porpoise/km$^2$, SE=0.21) and greatest in section D (2.3 porpoise/km$^2$, SE=0.74; Table 1). There were an estimated 299 harbor porpoise (CI=219–409) in all sections (Table 1), ranging from 30 harbor porpoise (CI= 5–60) in section A to 116 harbor porpoise (CI=62–221) in section D (Table 1). The pooled estimate of harbor porpoise abundance (299 porpoise) for all sections yielded the same abundance estimate as adding the estimates for each individual strata (A–E; Table 1).

If the present surveys were conducted annually with a similar sampling regime that produced an equally low CV (0.159), there would be sufficient power (80%) to detect a 14% annual change ($\alpha$=0.05; 12% change for $\alpha$=0.10) after five years.

Mean number of harbor porpoise per survey was greatest in section D and least in section E (Fig. 5A). Mean depth throughout the study area was 108.1 m (SE=21.68, $n$=584); section D had the greatest mean depth and section E the least (Fig. 5B). Harbor porpoise were distributed over a depth range of 20.1 to 235.0 m (mean=141.6 m, SE=2.43, $n$=275), with 83% of harbor porpoise sightings occurring over depths greater than 100 m. Significantly ($P$<0.05) fewer than expected harbor porpoise occurred in depths less than 75 m and significantly ($P$<0.05) more than expected in depth categories greater than 100 m (Fig. 6). The effect size (degree to which depths differed among categories) was small and the power to detect a difference was

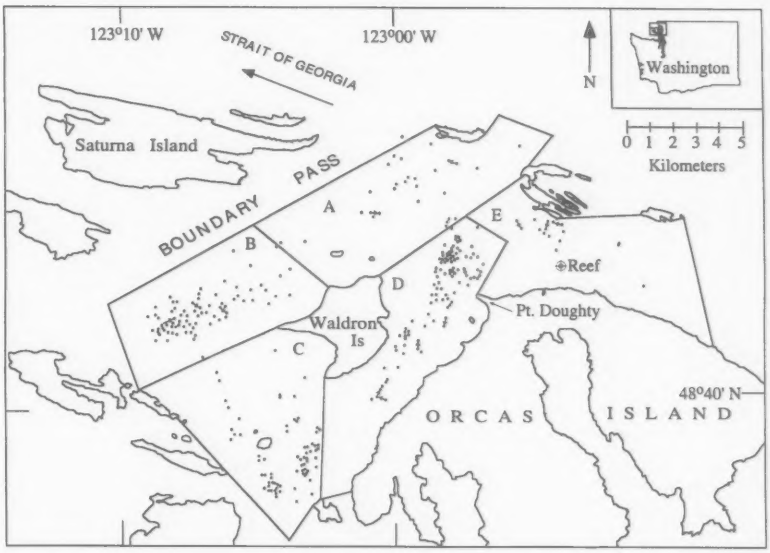


**Figure 3**

Location of 301 harbor porpoise sightings made during 73 random boat surveys completed from June to October 1992 within five stratified sections (A, B, C, D, E).

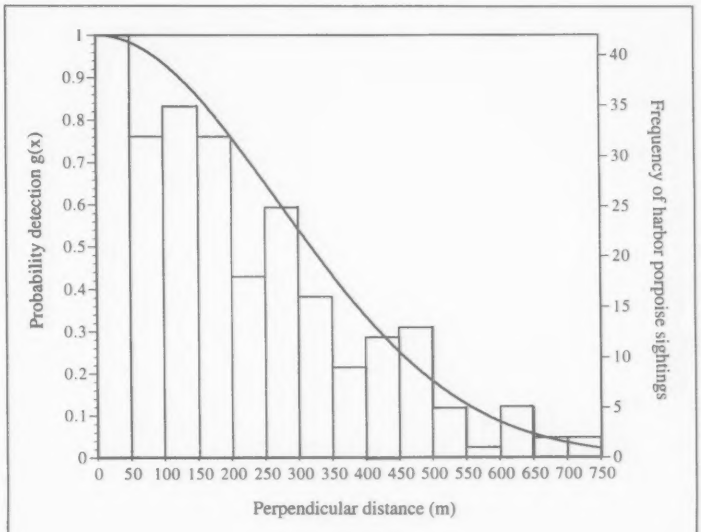


**Figure 4**

Probability detection distribution for perpendicular sighting distances of harbor porpoise. Bars represent frequency of sightings and the line represents the half-normal (hermite) model of best fit.

**Table 1**

Survey effort, line transect model parameters, density, and abundance estimates of harbor porpoise for each section (A–E) and all sections combined surveyed within the northern San Juan Islands, Washington, from June to October 1992.

| Parameter | Section A | Section B | Section C | Section D | Section E | All sections |
|---|---|---|---|---|---|---|
| Area ($km^2$) | 50.26 | 43.12 | 50.40 | 50.47 | 42.43 | 237 |
| Effort (km) | 112 | 120 | 120 | 112 | 96 | 560 |
| Transect lines | 14 | 15 | 15 | 14 | 12 | 70 |
| Truncation width (m) | 750 | 750 | 750 | 750 | 750 | 750 |
| Probability density $f(0)$/km | 2.96 | 2.96 | 2.96 | 2.96 | 2.96 | 2.96 |
| Sightings of harbor porpoise | 26 | 62 | 67 | 80 | 15 | 250 |
| Mean group size | 2.19 | 1.94 | 1.81 | 1.90 | 1.80 | 1.91 |
| Standard error (SE) of group size | 0.32 | 0.15 | 0.11 | 0.11 | 0.26 | 0.07 |
| Density (porpoise/$km^2$) | 0.60 | 1.03 | 1.55 | 2.33 | 0.76 | 1.26 |
| 95% confidence intervals (porpoise/$km^2$) | 0.30–1.21 | 0.60–1.75 | 0.77–3.10 | 1.24–4.4 | 0.35–1.66 | 0.92–1.73 |
| SE of density | 0.21 | 0.27 | 0.52 | 0.74 | 0.30 | 0.20 |
| % coefficient of variation (CV) of density | 35.09 | 26.00 | 33.86 | 31.55 | 39.16 | 15.86 |
| Abundance | 30 | 44 | 77 | 116 | 32 | 299 |
| 95% confidence interval (abundance) | 15–60 | 26–75 | 39–155 | 62–221 | 15–70 | 217–406 |
| SE of abundance | 10.53 | 11.43 | 26.07 | 36.91 | 12.53 | 47.0 |

low (37%) for categories that were not significantly different (Fig. 6). Given the small effect size, a power of 80% would require 358 locations of harbor porpoise in these three depth categories (there were 122 in this study). It is, therefore, unlikely that harbor porpoise occur in depths within these nonsignificant categories in different proportions than those available.

Mean seafloor slope for all sections combined was 9.85% (SE=0.656, $n$=584). Section B had the least slope and section C the greatest (Fig. 7). Harbor porpoise were sighted over a mean slope of 6.90% (SE=0.51, $n$=275, range: 0.37% to 45.75%). The greatest number of harbor porpoise sightings (79%) occurred over shallow slopes (<10%). There were significantly ($P$<0.05) greater numbers of harbor porpoise sightings than expected in category 0 to 2% slope, and significantly ($P$<0.05) fewer numbers of harbor porpoise than expected in categories 6 to 8%, 18 to 20%, and >26% slope (Fig. 8). The power to detect a difference was fairly high (69%) for categories that were not significantly different (Fig. 8). To increase power to 80%, we would need 151 samples within these ten categories (we had 126 samples).

Mean sea surface temperature (SST) recorded during all transects was 12.6°C (SE=0.081, $n$=427, range: 10.1° to 17.5°C). Little variability was found among the five sections. Section E had the greatest mean SST (mean=13.5°C, SE=0.22, $n$=69) and section B the least (mean=12.3°C, SE=0.14, $n$=97). Mean SST recorded during harbor porpoise sightings was 12.1°C (SE=0.09, $n$=267, range: 10.1° to 16.3°C). Harbor porpoise were sighted more frequently than expected ($P$<0.05) in water temperatures of 11° to 12°C and less frequently than expected ($P$<0.05) in water temperatures >16° C (Fig. 9). The power to detect a difference was moderate (50%) for categories that were not significantly different (Fig. 9). To increase power to 80%, we would need 180 samples within these ten categories (we had 171 samples).

There was no significant difference between number of harbor porpoise observed per minute during flood and ebb tides ($U$=315.5, $n$=69, $P$=0.076, $\alpha$=0.05). Bootstrap estimates (resampling statistics; 10,000 iterations) indicated an 86% chance of correctly rejecting the null hypothesis that mean number of sightings was equal between flood and ebb tides. Fifty samples in each tide stage (we sampled 52 in flood and 17 in ebb tide) were required to reject the null hypothesis at $\alpha$ = 0.05.

Mean Secchi reading for all harbor porpoise sightings was 9.3 m (SE=0.09, $n$=275, range: 5.7 to 11.9 m).

Greatest numbers of harbor porpoise were observed in mid-morning (1000 h) and afternoon (1400 to 1500 h) throughout the study area (Fig. 10). Fewer harbor porpoise were observed at midday (1100 to 1300 h; Fig. 10), although there was no significant difference ($H$=10.99, $n$=274, $P$=0.276) among mean number of harbor porpoise observed per minute and each hour of daylight surveyed (0900 to 1800 h).

Density estimates were calculated over all four months of the survey period rather than by month, which would have yielded too low of a sample size. If abundance estimates of porpoise had varied greatly among months during our survey period, we should have observed a higher CV (ours was relatively low: 0.159).

### Fixed boat surveys

Fifty-six sightings of 92 harbor porpoise were recorded during 33 surveys (port or starboard) in 1991, and 69 sightings of 118 harbor porpoise during 24 surveys (both sides of vessel surveyed) in 1992 (Fig. 2). Harbor porpoise were sighted during 79% of surveys in 1991 and 75% in 1992. Mean group size was 1.6 harbor porpoise (SE=0.09, $n$=56) in 1991 and 1.7 harbor porpoise (SE=0.127, $n$=69) in 1992. Distribution of harbor porpoise was patchy but similar between 1991 and 1992 (Fig. 2).

The greatest number of harbor porpoise sightings recorded per survey were along transects 1, 2, and 5 in 1991 and transects 1 and 5 in 1992 (Table 2; Fig. 2). The least number of sightings were recorded for transects 3 and 6 in 1991 and 1992 (Table 2; Fig. 2). Mean number of harbor porpoise sightings per survey was not significantly different ($P$>0.05) between 1991 and 1992 for any of the fixed transects (Table 2). Sample sizes for all transects were low because of the limited survey period (July to August). Given our low sample size (Cohen, 1988, requires a sample size of eight or more), we were unable to determine power. If eight samples of each fixed transect line had been taken, power to detect a difference in density between 1991 and 1992 would still have been low (power<27% for all fixed transects except transect three which had<6% power).

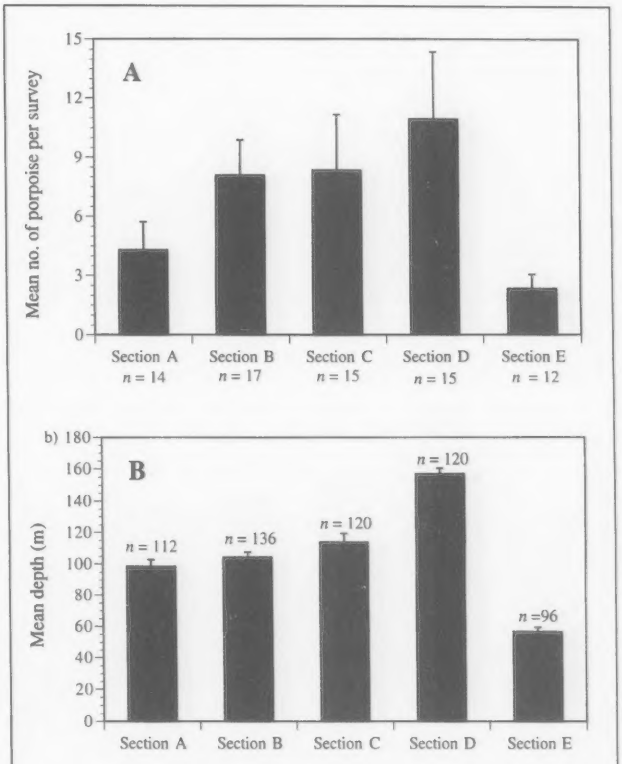


**Figure 5**

Mean number of harbor porpoise (**A**) and mean water depth (**B**) of each section (A–E) determined during random boat surveys (June–October 1992). Vertical lines represent standard error and "$n$" represents number of transects completed in each section (A; total=73) and random depth locations plotted within each section (B); total=584).

## Discussion

Population and density estimates of harbor porpoise were based on several assumptions of line transect theory. Relevant assumptions included the following: 1) study area was sampled randomly (transect lines placed randomly with respect to the distribution of objects) or animals were randomly distributed; 2) all animals on the trackline were detected; 3) group size was estimated without error; 4) locations were measured accurately for each individual or group; and 5) animals did not move in response to the survey vessel or were detected before they moved (Burnham et al., 1980).

The first assumption of line transect theory was met by employing a stratified random sampling design within the study area. This design was chosen so that the 8-km transects would adequately cover the entire study area. By using fixed length straight transects, however, certain areas of sections B and C were not adequately sampled. The habitat features of these areas were similar to the rest of the study area, and portions of section C not sampled during random surveys were sampled during fixed transect surveys 5 and 6. A study design that incorporated shorter transects (4 km) would allow more complete coverage of all areas within strata. If this study were replicated, we recommend incorporating 4-km transects to cover the areas that we missed. We do not believe, however, that our study design affected the results of the habitat correlates. By randomly surveying within a defined region off the northern San Juan Islands, we adequately sampled oceanographic features of interest (depth, seafloor slope, surface temperature, tides).

The assumption that all animals are detected on the trackline is often violated during marine mammal surveys. Animals with long durations of submer-

gence have a high probability of remaining undetected during the passage of an aircraft or vessel, resulting in availability bias (Marsh and Sinclair, 1989). Several studies (e.g. Marsh and Sinclair, 1989) of harbor porpoise have indicated, on the basis of perception bias, that the probability of detecting a harbor porpoise on the trackline, $g(0)$, is less than one (Barlow, 1988; Palka, 1993; Calambokidis[3]). Using an independent team of three observers, Barlow (1988) reported an estimated 22% of harbor porpoise that surfaced on the trackline were missed by a team of five observers (perception bias) traveling on a vessel at 18.5 km/h. Using three observers per survey, Calambokidis[3] and Palka (1993) estimated the probability of observing a group of harbor porpoise on the trackline, $g(0)$, was less than 0.5. We assumed $g(0)$ was one during our study because we were unable to determine availability or perception bias. It is probable that some porpoise did avoid the vessel and might have been submerged for up to five minutes (Raum-Suryan, 1995). It is, therefore, likely that $g(0)$ is less than one and harbor porpoise abundance is underestimated.

The ability to estimate group size can vary by the number of animals within the group and by the species of interest. Data from land-based calibration studies off the Washington coast indicated that observers on ves-

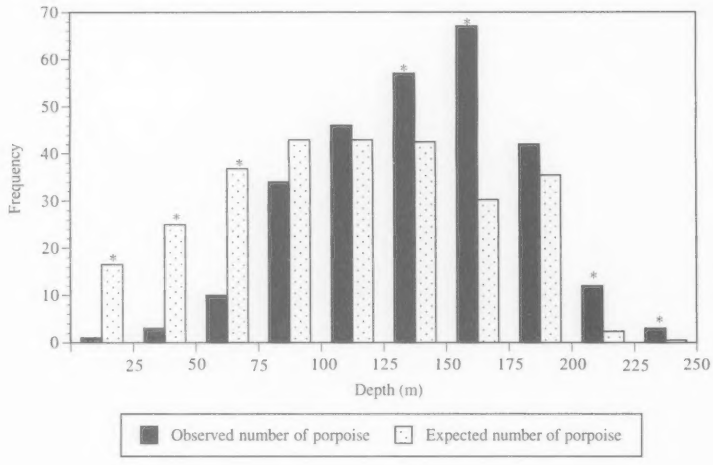


**Figure 6**

Depth distribution of 275 harbor porpoise sightings determined from random boat surveys (June to October 1992) in relation to expected distribution of harbor porpoise if they were distributed randomly with depth (as determined from depths at 584 random locations). An asterisk (*) designates a significant ($P<0.05$) difference determined with chi-square goodness-of-fit analyses.

[3] Calambokidis, J. 1991. Vessel surveys for harbor porpoise off the Washington coast. *In* H. Kajimura (ed.). Harbor porpoise interactions with Makah Salmon set net fishery in coastal Washington waters, 1988–89. National Marine Mammal Laboratory Processed Report, National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115.

**Table 2**

Mean, standard error (SE), number of sightings, and number of harbor porpoise determined during fixed boat surveys in 1991 and 1992. In 1992, the number of harbor porpoise observed (Obs.) are presented, as are values from four randomly chosen surveys used in analysis (Anal.), comparing mean number of harbor porpoise sighted along each transect in 1991 and 1992). *n* refers to the number of transect "sides" (bow out to 90° on port or starboard) surveyed.

| | 1991 | | | | | 1992 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mean | | SE | | *n* | | No. of sightings | | No. of porpoise | | |
| Transect no. | Mean | SE | *n* | No. of sightings | No. of porpoise | Obs. | Anal. | Obs. | Anal. | Obs. | Anal. | Obs. | Anal. | Obs. | Anal. | Probability |
| 1 | 2.20 | 0.80 | 5 | 11 | 18 | 2.50 | 2.00 | 0.42 | 0.41 | 8 | 4 | 20 | 8 | 31 | 13 | $P = 0.843$ |
| 2 | 1.83 | 0.48 | 6 | 11 | 16 | 1.25 | 1.25 | 0.45 | 0.63 | 8 | 4 | 10 | 5 | 22 | 17 | $P = 0.474$ |
| 3 | 1.00 | 0.38 | 7 | 7 | 13 | 0.13 | 0.25 | 0.13 | 0.25 | 8 | 4 | 1 | 1 | 1 | 1 | $P = 0.200$ |
| 5 | 2.83 | 0.54 | 6 | 17 | 29 | 2.25 | 3.00 | 0.49 | 0.58 | 8 | 4 | 18 | 12 | 25 | 18 | $P = 0.844$ |
| 6 | 0.60 | 0.40 | 5 | 3 | 6 | 0.88 | 0.50 | 0.40 | 0.29 | 8 | 4 | 7 | 2 | 12 | 3 | $P = 0.853$ |
| 7 | 1.75 | 0.63 | 4 | 7 | 10 | 1.50 | 1.00 | 0.82 | 0.41 | 8 | 4 | 12 | 9 | 26 | 19 | $P = 0.356$ |

sels underestimated true group size of harbor porpoise, missing up to 60% or more of animals (Calambokidis et al.[4]). However, these results were based on a very small sample size and the survey vessel traveled at twice the speed of our vessel. In our study, mean group size (1.91 porpoise) was only 5% different from that detected from concurrent shore-based surveys (2.01 porpoise; Raum-Suryan, 1995). We are, therefore, confident that any biases in group size estimates are small.

Accurately measuring the locations of marine mammals from vessels can be affected by the height of observers above water (Polacheck and Smith, 1989) and the use of reticle and compass binoculars (Smith, 1982; Barlow and Lee, 1994). From a low height above the water, angles are greatly affected by small deviations in reticle estimates. As radial distances to harbor porpoise decrease, however, errors in reticle estimates (sighting angles) have progressively less effect on distance calculations. The majority (78%) of our radial and perpendicular sighting distances were less than 350 m. At 350 m an error of ±0.1 reticle was equal to 50 m (the range of our data groupings which best fitted the model). Therefore, although the platform height of our survey vessel was low (2.68 m), we were able to obtain accurate sighting data by conducting surveys only during optimal sighting conditions (Beaufort ≤1), and because there was both a lack of ocean swell and the majority of sightings were less than 350 m distant.

Harbor porpoise are small, inconspicuous animals that avoid boats (Amudin and Amudin, 1974; Gaskin, 1977; Prescott and Fiorelli, 1980; Barlow, 1988). Detection of harbor porpoise before they become aware of the survey vessel is often difficult without prior knowledge of their locations. Polacheck and Thorpe (1990) observed harbor porpoise swimming away from their survey vessel a significant

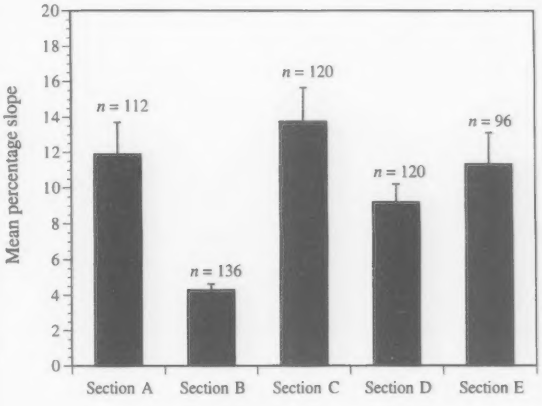


**Figure 7**

Mean percentage slope of seafloor for each section (A– E) determined from random boat surveys. Vertical lines represent standard error and "*n*" represents the number of random slope locations plotted within each section (total=584).

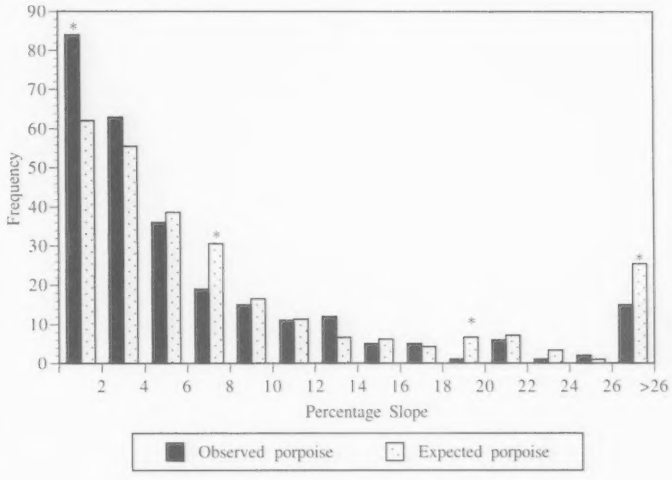


**Figure 8**

Harbor porpoise sightings (*n*=275) from random boat surveys (June–October 1992) in relation to expected distribution if porpoise were distributed randomly with slope (as determined from slopes at 584 random locations). An asterisk (*) designates a significant ($P<0.05$) difference, determined with chi-square goodness-of-fit analyses.

[4] Calambokidis, J., S. R. Melin, and D. J. Rugh. 1991. Land-based calibrations of harbor porpoise sightings from a vessel along the northern Washington coast. *In* H. Kajimura (ed.), Harbor porpoise interactions with Makah Salmon set net fishery in coastal Washington waters, 1988–89. National Marine Mammal Laboratory Processed Report. National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115-0070.

proportion of time. Barlow (1988) reported that harbor porpoise quickly avoid a closely approaching survey vessel. Vessel avoidance by harbor porpoise may result in animals remaining undetected by observers or may affect estimates of perpendicular distance of harbor porpoise from the vessel. If the frequency of harbor porpoise sightings were greatest near the trackline and decreased with increasing perpendicular distance during this study, it appeared that most harbor porpoise were detected before potentially significant vessel avoidance occurred. If porpoise did avoid the vessel, our abundance estimates would be underestimated. Ship avoidance was likely constant throughout the survey period, however, and would not have affected results of habitat correlates.

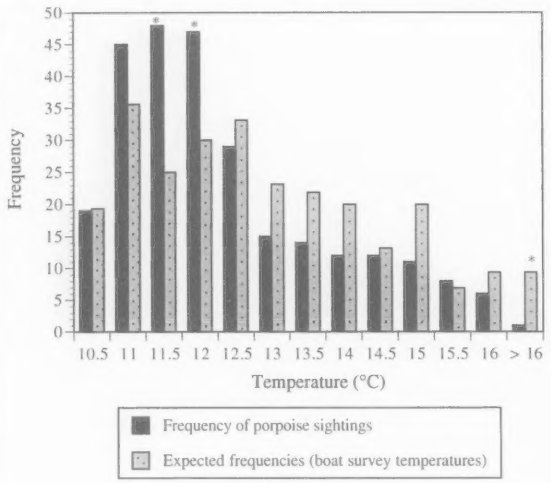


**Figure 9**

Sea surface temperatures (°C) recorded at harbor porpoise sightings ($n$=267) in relation to expected distribution of harbor porpoise if they were distributed randomly with temperature (as determined from temperatures at 427 locations along 73 random transect lines). An asterisk (*) designates a significant ($P$<0.05) difference, determined using chi-square goodness-of-fit analyses.

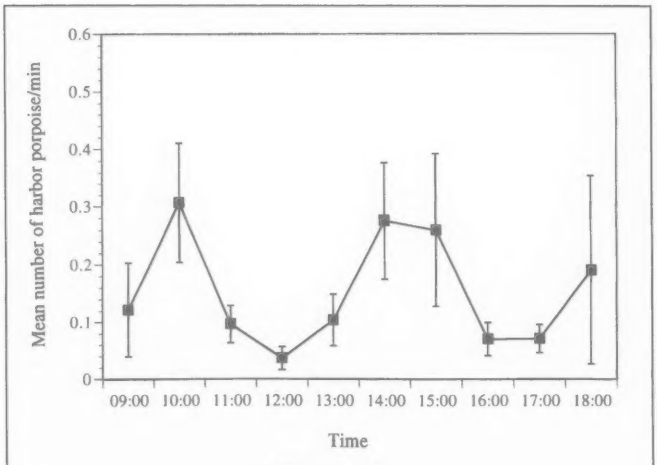


**Figure 10**

Mean number of harbor porpoise observed per minute during random boat surveys (June to October 1992) for all sections (A–E) combined. Vertical lines represent standard error.

Density estimates of harbor porpoise (1.26 porpoise/km$^2$) within the study area (237 km$^2$) were greater than densities reported by Calambokidis et al.[2] (0.42 porpoise/km$^2$) for waters off the San Juan Islands and part of the Strait of Georgia (2291 km$^2$) but were similar to density estimates of Flaherty and Stark[1] (0.85 to 1.63 porpoise/km$^2$) for the north and west San Juan Islands (1005 km$^2$). Density estimates reported by Calambokidis et al.[2] were based on an initial $g(0)$ equal to 0.324 (CV= 0.171) multiplied by a correction factor of 3.1 and yielding a $g(0)$ of one. Green et al.[5] surveyed an extensive area within the 100-m isobath off the coast of Oregon and Washington and also reported a much lower density of harbor porpoise (0.17 porpoise/km$^2$) than reported here. These differences probably result from Green et al.[5] and Calambokidis et al.[2] including regions of high and low harbor porpoise abundance in contrast to our focus on high density areas off the northern San Juan Islands.

Prey or habitat requirements often limit distribution of cetaceans to regions that may vary daily, seasonally, or yearly, depending on an individual's foraging, mating, or behavioral requirements. During this study, surveys were conducted only within the summer months (June to October) and thus may account for the relatively high density estimates of harbor porpoise within our study area. Flaherty and Stark[1] sighted harbor porpoise during all months of the year off the San Juan

[5] Green, G. A., J. J. Brueggeman, C. E. Bowlby, R. A. Grotefendt, M. L. Bonnell, and K. T. Balcomb III. 1992. Cetacean distribution and abundance off Oregon and Washington, 1989–1990. *In* J. J. Brueggeman (ed.), Final report prepared by Ebasco Environmental and Ecological Consulting, Inc. for Minerals Management Service, Pacific OCS Region. Offshore Continental Shelf (OCS) Study MMS 91-0093, 100 p.

Islands but observed more harbor porpoise in summer months (June to August) than other times of the year. Surveys of harbor porpoise throughout the year along the east and west coasts of the United States have indicated a seasonal pattern among various regions (Neave and Wright, 1968; Gaskin and Watson, 1985; Barlow, 1988; Green et al.[5]). Results of fixed transect surveys conducted in our study indicated no change in distributions of harbor porpoise between July and August 1991 and 1992. Clumped distribution of harbor porpoise along tracklines was likely associated with habitat features (harbor porpoise were sighted most often over deep water).

Among island regions, such as the Bay of Fundy, Glacier Bay, Alaska (Taylor and Dawson, 1984), and off the San Juan Islands, harbor porpoise are more often associated with deeper waters than along coastal regions of North America. Most harbor porpoise observed off the coast of California, Oregon, and Washington occurred at shallow water depths, and sightings decreased with increasing depth (Barlow, 1988; Dorfman, 1990; Calambokidis[3]; LaBarr and Ainley[6]). Incidental net entanglement of harbor porpoise within Washington waters occurred at the bottom of nets, at depths of 73 to 81 m (Scheffer and Slipp, 1948), and near the bottom or in the lower one-half of nets set from 11 to 18 m deep, indicating porpoise were foraging along the bottom or in deeper areas of the net (Gearin et al.[7]). The depth of water where harbor porpoise were sighted in this study may have been due to occurrence of prey within these areas.

The Pacific herring (*Clupea pallasi*) population in the Strait of Georgia is the largest known in Washington state, and herring are quite abundant in sections of the eastern Strait during summer, fall, and winter (Lemberg, 1978). During this study, harbor porpoise, harbor seals, and a minke whale were observed feeding on a school of Pacific herring. The dominant prey items in stomachs of harbor porpoise taken in a setnet fishery in summer off northern Washington were Pacific herring, market squid (*Loligo opalescens*), gadids, and osmerids (Gearin and Johnson[8]). Pacific herring and market squid migrate vertically within the water column, remaining close to the seafloor during the day, and approach the surface at night (Hart, 1973; Blaxter, 1985; Flaherty and Stark[1]). Characteristics of these prey items and the greater occurrence of porpoise over deep waters may indicate that harbor porpoise feed in deep water during the day. Aggregations of surface schooling fish and associated harbor porpoise were rarely (1% of surveys) observed within our study area, further indicating that harbor porpoise were likely feeding on prey in deep water.

In our study, harbor porpoise were sighted most often in shallow sloping areas with little bathymetric relief. These results contrast with those of Flaherty and Stark,[1] in which 70% of harbor porpoise sighted were found in areas with seafloor relief greater than 40%, and with those of Calambokidis,[3] who observed significantly more harbor porpoise than expected within areas of uneven bottom topography off the outer Washington coast. It is likely that slope of the seafloor does not significantly affect the distribution of harbor porpoise or their prey in our study area. We believe that harbor porpoise and their prey are associated with deeper waters in this region which, in general, has shallow slopes.

Water temperature may influence the distribution of harbor porpoise. Calambokidis[3] reported harbor porpoise sightings in water temperatures ranging from 9° to 16°C off Washington. In the Bay of Fundy, Watts and Gaskin (1985) found a negative correlation between harbor porpoise abundance and mean August SST, and Watson (1981) reported that harbor porpoise occurred in water temperatures less than 15°C in the Bay of Fundy. It is unlikely, however, that SST alone would influence harbor porpoise distribution. Most harbor porpoise entered Fish Harbor, New Brunswick, Canada, when SST was between 9° and 10°C, a period when large numbers of juvenile herring were also entering the region (Gaskin and Watson, 1985). Within the Bay of Fundy, Watts and Gaskin (1985) found herring associated with vertically mixed waters and reduced surface temperatures. This association was possibly due to increased concentrations of zooplankton, which also occurred along convergent zones (Watts and Gaskin 1985). Sea surface temperatures off the northern San Juan Islands, therefore, were possibly related to tidal currents that may be associated with concentrations of harbor porpoise prey.

[6] LaBarr, M. S., and D. G. Ainley. 1985. Depth distribution of harbor porpoise off central California: a report of cruises in April and May–June 1985. NMFS Contract No. 41 USC 252, 23 p.

[7] Gearin, P. J., M. A. Johnson, and S. Joner. 1991. Harbor porpoise interactions with the Makah Chinook Salmon Set-Net Fishery, 1988–89. *In* H. Kajimura (ed.), Harbor porpoise interactions with Makah salmon set net fishery in coastal Washington waters, 1988–89. National Marine Mammal Laboratory Processed Report. National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115-0070.

[8] Gearin, P. J., and M. A. Johnson. 1991. Prey identified from stomachs of harbor porpoise and Chinook salmon from the 1988–89 Makah Salmon Set-Net Fishery. *In* H. Kajimura (ed.), Harbor porpoise interactions with Makah Salmon set net fishery in coastal Washington waters, 1988–89. National Marine Mammal Laboratory Processed Report. National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115-0070.

In this study, SST was measured along tracklines and may not have represented water temperatures where harbor porpoise were sighted. Tide rips mixing water, or currents moving through the study area could have altered water temperatures by a few degrees between trackline and harbor porpoise locations. Sea surface temperature varied by 5°C from beginning to end of the 8-km tracklines. We assumed, however, less bias was introduced by collecting SST along the trackline than by continuously going off transect and potentially disturbing harbor porpoise ahead of the vessel. Because our methods were consistent over the study period, the comparison in use versus availability of SST is likely representative.

It is doubtful that time of day had a significant effect on the ability to sight harbor porpoise in our study; therefore, other environmental factors must have affected harbor porpoise distribution in relation to time of day. Occurrence of harbor porpoise appears closely associated with the strength of tidal currents. From shore-based surveys within our study area (Raum-Suryan, 1995), mean number of porpoise observed per minute was greatest two hours before each peak in the maximum flood tide, and significantly more ($P<0.05$) porpoise were observed per minute during flood than ebb tides. From June to October 1992, the majority of low tides in the northern San Juan Islands occurred in the early morning hours. The relation between the occurrence of harbor porpoise with tide and time of day indicates that porpoise movements may have been associated with concentrations of prey in flood currents and tide rips. It is possible that harbor porpoise range throughout Washington Sound but continue to return to northern San Juan Island waters as a primary foraging area.

We found a large proportion of the harbor porpoise population of Washington Sound located within our study area. Calambokidis et al.[2] estimated the population size of harbor porpoise for the San Juan Islands (2291 $km^2$) at 960 animals (corrected as in density estimate). In approximately 10% (237 $km^2$) of the area that Calambokidis et al.[2] surveyed, we estimated 30% (299 porpoise) of the harbor porpoise population. Given that our $g(0)$ was assumed to be one (thus underestimating the population size), the proportion of harbor porpoise within our study area is likely greater than 30% of the total population within the San Juan Island region. On the basis of pollutants detected in harbor porpoise tissues, porpoise along the west coast do not mix freely between California, Oregon, and Washington (Calambokidis and Barlow, 1991). In addition, Washington and California are considered repositories of genetic diversity for harbor porpoise of the Northeast Pacific (Rosel et al., 1995) and also indicate that harbor porpoise ranges may be restricted. In addition to aerial surveys conducted over Washington waters (Calambokidis et al.[2]), our study area appears to be an important site for monitoring trends in distribution and abundance of harbor porpoise in inland water of Washington.

It is not clear why harbor porpoise are not as abundant in other areas of Washington Sound as they are in our study area. The relatively low abundance outside our study area may be due to factors other than food availability, such as pollution, fishing pressure, increased boat traffic, or other environmental changes. We believe that harbor porpoise are more abundant in our study area than in other parts of Washington Sound because certain environmental conditions (deep, cool water, and strong tidal mixing) influence the distribution of harbor porpoise prey. Future monitoring studies on oceanographic conditions and prey availability associated with harbor porpoise sightings would greatly assist in determining mechanisms affecting harbor porpoise abundance and distribution in this and other areas and help in managing this genetically important stock.

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## Literature cited

**Amudin, M., and B. Amudin.**
**1974.** On the behaviour and study of the harbour porpoise, *Phocoena phocoena*, in the wild. *In* G. Pilleri (ed.), Inves-

tigations on Cetacea, vol. V, p. 317–328. Hirnanatomische Institut der Universität, Berne.

**Barlow, J.**
**1988.** Harbor porpoise, *Phocoena phocoena*, abundance estimation for California, Oregon, and Washington: I. Ship surveys. Fish. Bull. 86(3):417–432.

**Barlow, J., and T. Lee.**
**1994.** The estimation of perpendicular sighting distance on SWFSC research vessel surveys for cetaceans: 1974 to 1991. U.S. Dep. Commer., NOAA Tech. Memo.NMFS, NOAA, La Jolla, CA, 92038, 46 p.

**Blaxter, J. H. S.**
**1985.** The herring: a successful species? Can. J. Fish. Aquat. Sci. 42 (suppl. 1):21–30.

**Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake.**
**1993.** Distance sampling: estimating abundance of biological populations. Chapman and Hall, New York, NY, 446 p.

**Burnham, K. P., D. R. Anderson, and J. L. Laake.**
**1980.** Estimation of density from line transect sampling of biological populations. Wild. Monogr. 72:1–202.

**Calambokidis, J., and J. Barlow.**
**1991.** Chlorinated hydrocarbon concentrations and their use for describing population discreteness in harbor porpoises from Washington, Oregon, and California. *In* J. E. Reynolds III and P. K. Odell (eds.), Marine mammal strandings in the United States: proceedings of the second marine mammal stranding workshop Miami, FL, Dec. 3–5, 1987, p. 101–110. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 98.

**Calambokidis, J., J. Peard, G. H. Steiger, J. C. Cubbage, and R. L. DeLong.**
**1984.** Chemical contaminants in marine mammals from Washington state. U.S. Dep. Commer., NOAA Tech. Memo., NOS OMS 6, 167 p.

**Calambokidis, J., S. M. Speich, J. Peard, G. H. Steiger, J. C. Cubbage, D. M. Fry, and L. J. Lowenstine.**
**1985.** Biology of Puget Sound marine mammals and marine birds: population health and evidence of pollution effects. U.S. Dep. Commer., NOAA Tech. Memo., NOS OMA 18, 159 p.

**Cohen, J.**
**1988.** Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Assoc., Publ., Hillsdale, NJ, 567 p.

**Dorfman, E. J.**
**1990.** Distribution, behavior, and food habits of harbor porpoises (*Phocoena phocoena*) in Monterey Bay. M.S. thesis, Moss Landing Marine Laboratories, Moss Landing, CA, 57 p.

**Everitt, R. D., C. H. Fiscus, and R. L. DeLong.**
**1980.** Northern Puget Sound marine mammals. EPA-600/7-80-139. National Marine Mammal Laboratory, Natl. Mar. Fish. Serv., NOAA, Seattle, WA, 134 p.

**Gaskin, D. E.**
**1977.** Harbour porpoise *Phocoena phocoena* (L.) in the western approaches to the Bay of Fundy 1969–75. Rep. Int. Whal. Comm. 27:487–492.

**Gaskin, D. E., and A. P. Watson.**
**1985.** The harbor porpoise, *Phocoena phocoena*, in Fish Harbour, New Brunswick, Canada: occupancy, distribution, and movements. Fish. Bull. 83(3):427–442.

**Gerrodette, T.**
**1987.** A power analysis for detecting trends. Ecology 68(5):1364–1372.

**Hart, J. L.**
**1973.** Pacific fishes of Canada. Fish. Res. Board Can. Bull. 180, 740 p.

**Kozloff, E. N.**
**1973.** Seashore life of Puget Sound, the Strait of Georgia, and the San Juan Archipelago. Univ. Washington Press, Seattle, WA, 282 p.

**Laake, J. L., S. T. Buckland, D. R. Anderson, and K. P. Burnham.**
**1993.** DISTANCE user's guide. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins, CO 80523, 84 p.

**Lemberg, N. A.**
**1978.** Hydroacoustic assessment of Puget Sound herring, 1972–1978. Washington Dep. Fisheries, Tech. Rep. 41, 43 p.

**Marsh, H., and D. F. Sinclair.**
**1989.** Correcting for visibility bias in strip transect aerial surveys of aquatic fauna J. Wildl. Manage. 53:1017–1024.

**Neave, D. J., and B. S. Wright.**
**1968.** Seasonal migrations of the harbor porpoise (*Phocoena phocoena*) and other cetacea in the Bay of Fundy. J. Mamm. 49(2):259–264.

**National Oceanic and Atmospheric Administration.**
**1991.** High and low water predictions: west coast of North and South America including the Hawaiian Island. U.S. Dep. Commer., NOAA, 238 p.

**Palka, D. L.**
**1993.** Estimating density of animals when assumptions of line-transect surveys are violated. PhD. diss., Univ. California, San Diego, CA, 69 p.

**Polacheck, T., and T. D. Smith.**
**1989.** A proposed methodology for field testing line transect theory for shipboard surveys of cetaceans. Rep. Int. Whal. Comm. 39:341–345.

**Polacheck, T., and L. Thorpe.**
**1990.** The swimming direction of harbor porpoise in relationship to a survey vessel. Rep. Int. Whal. Comm. 40:463–470.

**Prescott, J. H., and P. M. Fiorelli.**
**1980.** Review of the harbor porpoise (*Phocoena phocoena*) in the U.S. Northwest Atlantic. U.S. Dep. Commer., National Tech. Info. Ser., pb-80-176928, 64 p.

**Raum-Suryan, K. L.**
**1995.** Distribution, abundance, habitat use, and respiration patterns of harbor porpoise (*Phocoena phocoena*) off the northern San Juan Islands, Washington. M.S. thesis, Moss Landing Marine Laboratories, Moss Landing, CA, 79 p.

**Resampling Stats, Inc.**
**1995.** Resampling Stats users guide. Resampl. Stats., Inc., Arlington, VA, 128 p.

**Rosel, P. E., A. E. Dizon, and M. G. Haygood.**
**1995.** Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. Can. J. Fish. Aquat. Sci. 52:1210–1219.

**Scheffer, V. S., and J. Slipp.**
**1948.** The whales and dolphins of Washington State with a key to the cetaceans of the west coast of North America. Am. Midl. Nat. 39:257–337.

**Smith, T. D.**
**1982.** Testing methods of estimating range and bearing to cetaceans aboard the RV *P.S. Jordan*. U.S. Dep. Commer, NOAA Tech. Memo. NMFS, NOAA, La Jolla, CA 92038, 30 p.

**Smith, G. J. D., and D. E. Gaskin.**
**1983.** An environmental index for habitat utilization by female harbour porpoises with calves near Deer Island, Bay of Fundy. Ophelia. 22:1–15.

**Taylor, B. L., and P. K. Dawson.**
**1984.** Seasonal changes in density and behavior of harbor porpoise (*Phocoena phocoena*) affecting censusing methodology in Glacier Bay National Park, Alaska. Rep. Int. Whal. Comm. 34:479–483.

**Watson, A. P.**
**1976.** The diurnal behaviour of the harbour porpoise (*Phocoena phocoena* L.) in the coastal waters of the western Bay of Fundy. M.S. thesis, Univ. of Guelph, Ontario, Canada, 94 p.

**1981.** Sea guide to whales of the world. E.P. Dutton, New York, NY, 302 p.

**Watts, P., and D. E. Gaskin.**
**1985.** Habitat index analysis of the harbor porpoise (*Phocoena phocoena*) in the southern coastal waters of the Bay of Fundy. Can. J. Zool. 61(1):126–132.

**Zar, J. H.**
**1984.** Biostatistical analysis (second ed.). Prentice-Hall, Inc., Englewood Cliffs, NJ, 718 p.

# Global phylogeography of mackerels of the genus *Scomber*

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**Abstract.**—Inter- and intraspecific genetic relationships among and within three species of mackerels of the genus *Scomber* were investigated by restriction site analysis of the whole mitochondrial (mt) DNA genome and direct sequence analysis of the mitochondrial cytochrome *b* gene. A total of 15 samples, averaging 19 individuals each, were collected from geographically isolated populations throughout the ranges of *S. scombrus* (two samples), *S. australasicus* (five samples), and *S. japonicus* (eight samples). Restriction site analysis with 12 restriction enzymes revealed substantial genetic variation within each species. Sample haplotype diversities ranged from 0.28 to 0.95, and nucleotide sequence diversities from 0.13% to 0.76%. Spatial partitioning of genetic variation was observed in each of the species. Eastern and western North Atlantic samples of *S. scombrus* exhibited significant heterogeneity in the distribution of mtDNA haplotypes, but no fixed restriction site differences were observed between samples. Similarly, no fixed restriction site differences occurred among samples of *S. japonicus* in the Atlantic Ocean, although there were significant differences in the distribution of haplotypes among samples. In contrast, samples of *S. japonicus* from within the Pacific Ocean were characterized by fixed restriction site differences. North and South Pacific samples of *S. australasicus* were highly divergent, and one of two divergent mtDNA matrilines was restricted to samples from the South Pacific. A 420-bp segment of the cytochrome *b* gene was sequenced for representatives of each of the major mtDNA lineages identified by restriction site analysis. *Scomber scombrus* differed from *S. australasicus* and *S. japonicus* by more than 11% net nucleotide sequence divergence, considerably greater than the 3.5% sequence divergence between *S. australasicus* and *S. japonicus*. Levels of interspecific genetic divergences based on restriction site data were similar in pattern, but were approximately 20% lower in magnitude when based on the cytochrome *b* sequences. Parsimony analysis and neighbor-joining of restriction site data, and parsimony analysis of cytochrome *b* sequences showed similar paraphyletic patterns in both *S. japonicus* and *S. australasicus*. Levels of divergence among samples of *S. japonicus* were similar to those between samples of *S. australasicus* and *S. japonicus*. Complete partitioning of halpotypes among some samples of *S. japonicus* that are morphologically distinct suggests that Atlantic and Indo-Pacific populations of *S. japonicus* may need to be recognized as separate species.

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Population structure is largely influenced by the biological characteristics of a species and the attributes of its environment that promote or impede gene flow. In marine fishes, dispersal of planktonic early life history stages and adult vagility can facilitate genetic exchange over great distances (Rosenblatt, 1963; Shulman and Bermingham, 1995). Features of the marine environment that limit gene flow are often equally large in scale, such as current systems, major changes in temperature or salinity, or the presence of large land masses (Sinclair, 1988). Accordingly, many genetic analyses of broadly distributed marine fishes have shown little divergence among conspecific samples from geographically distant locations.

Limited population structure has been demonstrated for a variety of marine fishes. Electrophoretic analyses of allozymes have revealed little population divergence among collections of milkfish (*Chanos chanos*) separated by up to 10,000 km (Winans, 1980), damselfish, *Stegastes fasciolatus*, sampled from throughout the 2500 km Hawaiian archipelago (Shaklee, 1984), in twelve species of tropical marine shore fishes sampled from both sides of the Pacific Barrier, a 5000-km expanse of deep ocean separating central and eastern Pacific shallow water habitats (Rosenblatt and Waples, 1986), or in five species of damselfishes collected from isolated Caribbean reefs (Lacson, 1992 ). Similarly, analyses of mitochondrial DNA have revealed little genetic differentiation among populations of five species of tropical reef fishes sampled from throughout the Caribbean (Shulman and Bermingham, 1995), or within three cosmopolitan species of tunas (Graves et al., 1984; Graves and Dizon, 1989; Scoles and Graves, 1993).

Much less information is available on the population structure of broadly distributed marine species that occur in fragmented or disjunct distributions. Recent studies of striped mullet (*Mugil cephalus*,

Crosetti et al., 1994) and bluefish (*Pomatomus saltatrix*, Goodbred and Graves, 1996) have demonstrated that significant phylogeographic population structuring can exist in discontinuously distributed marine fishes. The reduced gene flow among isolated populations of such species provide excellent opportunities for studying the effects of historical marine zoogeographical processes and observe incipient speciation.

Mackerels of the genus *Scomber,* like the striped mullet and bluefish, are ideally suited for studies of genetic population structure in cosmopolitan fishes with fragmented distributions and for comparative studies that describe genetic patterns of other more vagile scombrids (Graves et al., 1984; Graves and Dizon, 1989; Scoles and Graves, 1993). Distributional patterns of scombrids vary widely from world-wide species such as the yellowfin tuna, albacore, and skipjack tunas to supposedly wide-spread species such as the chub mackerel, *S. japonicus*, which is divided into geographically disjunct populations, to those with limited ranges such as a Spanish mackerel (*Scomberomorus munroi*) found only in the Gulf of Papua between Australia and New Guinea (Collette and Nauen, 1983). The three species of *Scomber* (*S. japonicus, S. australasicus, and S. scombrus*) occur in temperate to subtropical waters, and two (*S. japonicus* and *S. australasicus*) display antitropical distributions. Each species occurs in disjunct populations of various sizes, and their distributions overlap in several areas (Fig. 1; Collette and Nauen, 1983). The cosmopolitan chub mackerel, *S. japonicus*, occurs in coastal regions and adjacent seas of the Atlantic, Pacific, and northwest Indian oceans. The spotted chub mackerel, *S. australasicus*, is restricted to the Pacific Ocean, southeastern Indian Ocean, and the Red Sea. The Atlantic mackerel, *S. scombrus*, is restricted to the North Atlantic Ocean. The present study emphasizes *S. japonicus*, which of the three species is the most widely distributed and morphologically divergent among its isolated populations (Matsui, 1967).

## Life histories and population structures

The spawning behavior and duration of the larval stage varies among the three species of *Scomber*. *Scomber scombrus* is capable of spawning serially up to 30 times in a spawning season (Watson et al., 1992) at any time of day (Walsh and Johnstone, 1992), where *S. japonicus* spawns on average only 9 times in a season, and does so only at night (Watanabe,

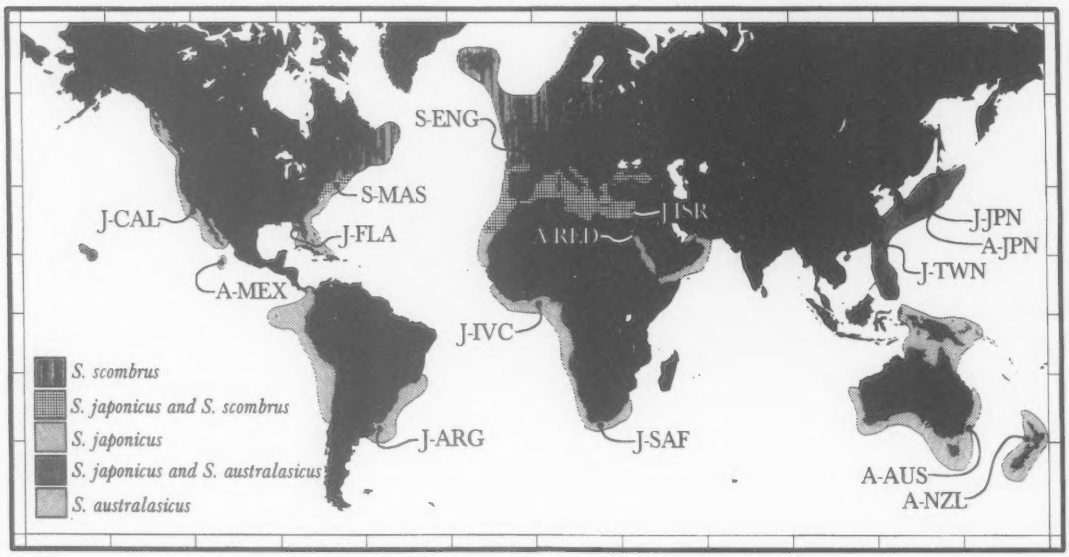



**Figure 1**

Sampling and distributions of Atlantic mackerel, *Scomber scombrus* (S-MAS and S-ENG), spotted chub mackerel, *S. australasicus* (A-RED, A-JPN, A-AUS, A-NZL, and A-MEX), and chub mackerel, *S. japonicus* (J-JPN, J-TWN, J-CAL, J-HAW, J-FLA, J-ARG, J-ISR, J-IVC, and J-SAF), according to Collette and Nauen (1983). *S. japonicus* distributions off Brazil and Namibia follow Perrotta and Aubone (1991) and Zenken and Lobov (1989), respectively.

1970). *Scomber japonicus* and *S. scombrus* have similar larval stage durations: eggs of *S. scombrus* and *S. japonicus* hatch in less than 6 d, and schooling behavior begins when larvae metamorphose (about 15 mm) which occurs at 24–9 d and 22–9 d, respectively (Hunter and Kimbrell, 1980; Ware and Lambert, 1985). Thus the duration of passive planktonic transport of the early life history stages is usually 29 d or less, but probably extends into the early juvenile stages as well. Little spawning or larval ecology data are available for *S. australasicus*, but it is expected that it has a similar early life history.

Exchange between regional populations of *S. scombrus* in the North Atlantic appears sufficient to maintain genetic homogeneity. Two spawning groups in the northwest Atlantic, the "northern contingent" in the southern Gulf of St. Lawerence, and the "southern contingent" between Cape Cod and Cape Hatteras, were identified by Sette (1950) by size composition and tagging data. However, these groups were not discriminated by meristic or growth character analysis (MacKay and Garside, 1969; Simard et al., 1992), or by allozyme analysis (Maguire et al., 1987). In the northeast Atlantic, two spawning groups, the "western stock" south and west of the British Isles, and the "North Sea stock," were identified by tagging (Hamre, 1980), but these groups were not distinguished by allozymic differences (Jamieson and Smith, 1987).

Matsui (1967) showed greater phenotypic variation in *S. japonicus* than in the other two species of *Scomber*, possibly because of its wider distribution. Populations of *S. japonicus* from the eastern and western Atlantic had nonoverlapping distributions of gill-raker counts, and other variable morphological characters of Atlantic populations were similar, including belly spots, strongly crenulated teeth, and large scales. In contrast, Pacific *S. japonicus* exhibited lightly crenulated teeth, no belly spots, and smaller scales (Matsui, 1967). Analysis of four polymorphic allozyme loci revealed no divergence among samples of *S. japonicus* from the southeast Atlantic off Namibia (Zenkin and Lobov, 1989), but heterogeneity in immunological reactivity suggested population structure off the northwest African coast (Weiss, 1980). A study of 14 polymorphic allozyme loci showed significant differences between samples from the north- and southeastern Pacific Ocean, which suggested reduced gene flow across the tropics in comparison with other similarly distributed pelagic fishes (Stepien and Rosenblatt, 1996). Differences in growth rates and morphological characters among samples within the southwest Atlantic led Perrotta (1993) to conclude that *S. japonicus* populations within the region had diverged to the level of subspecies.

To evaluate the genetic relationships among the three species of *Scomber*, and the discontinuous populations within them, we examined mitochondrial (mt) DNA restriction sites, and cytochrome *b* sequences. Analysis of mtDNA has proven useful in revealing phylogeographic structure in a variety of marine and freshwater fish species (Avise, 1992). Because mtDNA is clonally inherited, information regarding historical phylogenetic relationships is retained, hence analysis of mtDNA can render considerable information on historical relationships among populations and the mtDNA lineages they possess.

## Materials and methods

### Specimen collection

Samples of 15 to 21 individuals each of *S. scombrus*, *S. australasicus*, and *S. japonicus* were obtained from 15 locations (Table 1, Fig. 1). Specimens of *Rastrelliger kanagurta*, a species of the sister group to *Scomber* (Collette et al., 1984), were obtained from Sri Lanka. Whole fish were frozen after collection and shipped to the laboratory on dry ice.

### MtDNA preparation and analysis

MtDNA-enriched genomic DNA was isolated from 3 g of lateral red muscle, or whole young-of-year fish with digestive tracts removed (A-NZL only) according to Chapman and Powers (1984) method, modified by the omission of sucrose step gradients and the use of 1.5% sodium dodecyl sulfate for mitochondrial lysis. DNAs were digested with 10 hexameric (*Apa*I, *Bgl*I, *Bsu*36I, *Dra*I, *Pvu*II, *Sca*I, *Stu*I, *Ssp*I, *Hpa*I, *Spe*I) and 2 multi-hexameric (*Ava*I, *Hae*II) restriction endonucleases following manufacturers' (Stratagene and Gibco-BRL) instructions. DNA fragments were separated by electrophoresis in 1.0 or 1.5% agarose gels, transferred to nylon membranes by Southern transfer, and hybridized to a biotin-labeled probe as described previously (Scoles and Graves, 1993). The probe was made by separately cloning four yellowfin tuna, *Thunnus albacares*, mtDNA fragments that cover the entire mitochondrial genome in the *Pst*I site of pBluescript SK- (Stratagene). MtDNA fragments were visualized by using the BluGene Non-Radioactive Nucleic Acid Detection Kit (Gibco-BRL).

A 12-letter composite mtDNA haplotype, indicating the fragment pattern for each enzyme, was developed for each individual. Letters were assigned to restriction fragment patterns as they were encountered, beginning with 'A' for the closely related

**Table 1**

*Scomber japonicus*, *S. australasicus*, and *S. scombrus*. Sample name, size, location, haplotype diversity ($h$), and percent nucleotide sequence diversity ($\pi$).

| Sample name | Sample size ($n$) | Sample location | Haplotype diversity ($h$) | Percent nucleotide sequence diversity ($\pi$) |
|---|---|---|---|---|
| *Scomber scombrus* | | | | |
| S-MAS | 20 | Boston, Massachusetts | 0.85 | 0.29 |
| S-ENG | 20 | Plymouth, England | 0.28 | 0.07 |
| Total | 40 | | 0.58 | 0.18 |
| *Scomber australasicus* | | | | |
| A-RED | 15 | Sinai Peninsula south, Red Sea | 0.95 | 0.41 |
| A-AUS | 18 | New South Wales, Australia | 0.86 | 0.75 |
| A-NZL | 19 | Wellington, New Zealand | 0.75 | 0.77 |
| A-JPN | 21 | Tokyo, Japan | 0.81 | 0.30 |
| A-MEX | 20 | Revillagigedo Islands, Mexico | 0.59 | 0.13 |
| Total | 93 | | 0.90 | 2.18 |
| *Scomber japonicus* | | | | |
| J-FLA | 20 | Panama City, Florida | 0.93 | 0.40 |
| J-ARG | 18 | Mar del Plata, Argentina | 0.90 | 0.50 |
| J-ISR | 20 | Mediterranean coast of Israel | 0.90 | 0.38 |
| J-IVC | 20 | Abidjan, Ivory Coast | 0.91 | 0.39 |
| J-SAF | 20 | Cape Town, South Africa | 0.81 | 0.38 |
| J-TWN | 20 | Kaohsing, Taiwan | 0.86 | 0.29 |
| J-JPN | 20 | Tokyo, Japan | 0.88 | 0.35 |
| J-CAL | 20 | San Diego, California | 0.64 | 0.14 |
| Total | 158 | | 0.95 | 2.42 |
| Grand total | 291 | | | |

*S. japonicus* and *S. australasicus* group, and proceeding through the alphabet, and beginning with 'Z' and proceeding in reverse alphabetical order for *S. scombrus*. For the few *S. scombrus* restriction fragment patterns that also occurred in *S. japonicus* or *S. australasicus*, the latter species' restriction morph letter designation was used.

Estimates of nucleotide sequence divergence ($d$) among haplotypes were determined by using the approach of Nei and Li (1979) for fragment data, and Nei and Tajima (1981) and Nei and Miller (1990) for site data, with weighting based on the proportion of fragments or sites produced by each enzyme class (Nei and Tajima, 1983). Estimates of nucleon or haplotype diversity ($h$) and nucleotide sequence diversity ($\pi$) were then calculated with the program DA of the statistical package REAP (McElroy et al., 1992). Corrected nucleotide sequence divergences ($\delta$) among populations were calculated by using the protocol of Nei and Li (1979). The homogeneity of haplotype distributions among samples was evaluated by chi-square analyses using the Monte-Carlo method of Roff and Bentzen (1989) with 1000 randomizations of the data with REAP.

Restriction sites were inferred from completely additive fragment patterns, and the information for each individual was coded using a presence-or-absence matrix. Homology of restriction sites could not be assured between *S. scombrus* and the *S. australasicus-S. japonicus* group owing to the relatively large genetic divergence between the species. Restriction site data were therefore evaluated only for phylogenetic relationships within the *S. australasicus-S. japonicus* group, and an estimate of divergence between *S. scombrus* and the pooled data of *S. australasicus* and *S. japonicus* was determined by using restriction fragment data. Relationships among haplotypes were inferred by cluster analysis (unweighted pair-group method, UPGMA, Norusis, 1988). Neighbor-joining, parsimony analyses and consensus trees were generated with the PAUP* software program (test version).

## DNA amplification and sequence analysis

Individuals representative of the major *Scomber* mtDNA matrilines, identified by restriction site analysis, and of *Rastrelliger kanagurta*, were selected for DNA sequence analysis. A 418-bp region of the cytochrome *b* gene was amplified from mtDNA-enriched genomic DNA isolations by the polymerase chain reaction (PCR) with primers L15079

(5'GAGGCCTCTACTATGGCTCTTACC3') and H15497 (5'GCTAGGGTATAATTGTCTGGGTCGCC3') developed by Finnerty and Block (1992) for blue marlin (*Makaira nigricans*). Double-stranded DNA amplifications were accomplished in 100 µl of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM of each dNTP, 50 pmol of each primer, 50 ng genomic DNA, and 2.5 units *Taq* polymerase (Perkin-Elmer/Cetus). Cycling parameters were preceded by a 4-min initial denaturation at 94°C and included 38 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min, followed by a 5-min final extension at 72°C. Primers L15079 and H15497 would not amplify some haplotypes of *S. australasicus*. Specific primers with conserved 3' ends were designed from other sequences: CYT2A (5'TACCTTTTCATGGAAACATG3') and CYT2B (5'AAGAGGTTGGGAGAGAAGA3'). Both strands were fully sequenced by using the Sequenase kit (United States Biochemical) or the CircumVent Cycle Sequencing Kit (New England BioLabs) following the vendors' protocols.

Sequences were aligned by eye to the human mitochondrial cytochrome *b* sequence[1] (Anderson et al., 1981). Nucleotide sequence divergences (*d*) among cytochrome *b* sequences were generated with the program K of REAP. Cytochrome *b* sequences were analyzed by parsimony analysis with rooting to *Rastrelliger kanagurta* by using ALLTREES in PAUP.

## Results

MtDNA restriction site analysis of 40 *Scomber scombrus* from two locations revealed a total of 56 restriction sites, of which 16 were polymorphic, defining 13 haplotypes. Nucleotide sequence divergences among the haplotypes of *S. scombrus* ranged from $d$=0.17% to 0.86%. An average of 294 bp was surveyed, representing 1.75% of the mitochondrial genome. The average size of the *S. scombrus* mtDNA genome, determined from several restriction fragment profiles from each of the 12 restriction enzymes, was 16,784 ±213 bp (SD).

Restriction site analysis of 246 *S. japonicus* and *S. australasicus* revealed a total of 93 unique haplotypes, including information from 86 restriction sites, of which 58 were polymorphic. Among haplotypes of *S. japonicus* and *S. australasicus*, nucleotide sequence divergences ranged from $d$=0.15% to 2.9%. On average, 310 bp were surveyed per individual, representing 1.85% of the mtDNA genome. The average size of the mtDNA genome of *S. japonicus* or *S. australasicus* was estimated to be 16,781 ±195 bp (SD), not statistically different than that of *S. scombrus*.

### Genetic diversities

MtDNA restriction site analysis revealed that the genetic variability was lower in *S. scombrus* than the other two species (Table 1). S-ENG was the least variable sample in the study ($h$=0.28), consisting of four haplotypes of which three occurred only once (Table 2). Ten haplotypes were revealed in S-MAS, with three at elevated frequencies, resulting in a haplotype diversity of $h$=0.85. The overall haplotype diversity for *S. scombrus* was $h$=0.58, and nucleotide sequence diversity was $\pi$=0.18%. Three restriction enzymes, *Pvu*II, *Bgl*I, and *Bsu*36I, were invariant in *S. scombrus*, whereas the remaining nine restriction enzymes each revealed from two to five fragment patterns.

A wide range of diversities was observed among samples of *S. australasicus* ($h$=0.59–0.95) and *S. japonicus* ($h$=0.64–0.93) based on the restriction site data. Eastern Pacific samples of *S. australasicus* and *S. japonicus* from Mexico (A-MEX, $h$=0.59, $\pi$=0.13%) and California (J-CAL, $h$=0.64, $\pi$=0.14%) had lower diversities than other samples, possessing only four and six haplotypes, respectively (Tables 1 and 2). The highest haplotype diversity occurred in A-RED ($h$=0.95), with five of 10 haplotypes represented twice; the highest nucleotide sequence diversities occurred in A-NZL ($\pi$=0.77%) and A-AUS ($\pi$=0.75%) owing to the presence of two divergent mtDNA matrilines within each sample (Tables 1 and 2). With the exception of *Bgl*I in *S. australasicus*, all restriction enzymes were variable in both species, revealing two to seven fragment patterns in *S. australasicus*, and two to 15 in *S. japonicus*. The enzyme *Apa*I revealed the greatest number of variants in both species.

### Genetic divergences

Restriction fragment analysis showed *S. scombrus* to be highly divergent from *S. australasicus* and *S. japonicus*, with a net nucleotide sequence divergence of $\delta$=11.9% between *S. scombrus* and the pooled data of *S. japonicus* and *S. australasicus*. In comparison, the mean net nucleotide sequence divergence between *S. japonicus* and *S. australasicus* based on the restriction site data was only $\delta$=1.17%. Probabilities of Roff and Bentzen chi-square tests among samples that shared haplotypes are given in Table 3.

***Scomber scombrus*** Considerable intraspecific divergence was evident in *S. scombrus* from the mtDNA

[1] GenBank accession no. V00662.

**Table 2**

Distributions of mtDNA haplotypes among samples of species of *Scomber*, grouped by their occurrence in phenetic cluster analysis. Letters represent fragment patterns produced by the following enzymes (left to right): *Sca*I, *Dra*I, *Stu*I, *Pvu*II, *Hae*II, *Apa*I, *Ava*I, *Ssp*I, *Bgl*I, *Hpa*I, *Spe*I, *Bsu*36I.

Western Pacific *Scomber japonicus*

| ID | Haplotype | J-TWN | J-JPN | Total |
|---|---|---|---|---|
| 1 | BAIABIAGEBAA | 4 | 5 | 9 |
| 2 | BAIABLADEBAA | 5 | 4 | 9 |
| 3 | BAIABMAGEBAA | 5 | 4 | 9 |
| 4 | BAIABJADEBAA | 2 | 1 | 3 |
| 5 | BAIABMHGEBAA | | 2 | 2 |
| 6 | BAIABJAGEBAA | | 1 | 1 |
| 7 | BAIABLAGEBAA | 1 | | 1 |
| 8 | BAIABMADEBAA | 1 | | 1 |
| 9 | BAIABMAGEBCA | | 1 | 1 |
| 10 | BAIABNAGEBAA | | 1 | 1 |
| 11 | BAIABOAEEBAA | | 1 | 1 |
| 12 | BAIABOAGEBAA | 1 | | 1 |
| 13 | BAIAGIAGEBAA | 1 | | 1 |

Eastern Pacific *Scomber japonicus*

| ID | Haplotype | J-CAL | Total |
|---|---|---|---|
| 14 | BAIABIADEAAA | 12 | 12 |
| 15 | BAIABJADEAAA | 5 | 5 |
| 16 | BAIABIADEAAB | 1 | 1 |
| 17 | BAIABIDDEAAA | 1 | 1 |
| 18 | BAIADIADEAAA | 1 | 1 |
| 19 | BAJABIADCAAA | 1 | 1 |

Atlantic *Scomber japonicus*.

| ID | Haplotype | J-FLA | J-ARG | J-SAF | J-IVC | J-ISR | Total |
|---|---|---|---|---|---|---|---|
| 20 | ABAAABAAAAAA | 1 | | 1 | 3 | 1 | 6 |
| 21 | AAAAABADAAAA | 1 | | | 1 | | 2 |
| 22 | ABAAABAHAAAA | | 3 | | | | 3 |
| 23 | ABAAABADAAAA | | | | | 2 | 2 |
| 24 | ABAAABAIAAAA | | 1 | | | | 1 |
| 25 | ABAAADAAAAAA | | | 1 | | | 1 |
| 26 | ABAAAKADAAAA | | | | | 1 | 1 |
| 27 | ABAAEBAAAAAA | | | | 1 | | 1 |
| 28 | ABAAEBADAAAA | | | | | 1 | 1 |
| 29 | ABABABAHAAAA | | 1 | | | | 1 |
| 30 | AAAAABAAAAAA | 1 | | | | | 1 |
| 31 | AAAAAAAAAAAA | 6 | 4 | | 3 | 2 | 15 |
| 32 | AABAAAAAAAAA | 4 | | | | | 4 |
| 33 | AAAAAJAAAAAA | | 2 | | | | 2 |
| 34 | AAAAAAABAAAA | | | | 1 | | 1 |
| 35 | AAAAAPAAAAAA | | 1 | | | | 1 |
| 36 | AAAAAABAABAA | | | 1 | | | 1 |
| 37 | AABAAAAABAAA | 1 | | | | | 1 |
| 38 | AABAAAACAAAA | 1 | | | | | 1 |
| 39 | ABAAAAAAAAAA | 1 | | | | | 1 |
| 40 | BAAAAJAAAAAA | | 1 | | | | 1 |
| 41 | AAGAAAADAAAA | | 4 | 9 | 5 | 6 | 24 |
| 42 | ABGAAAADAAAA | | | 1 | 2 | 3 | 6 |
| 43 | AAAAAAADAAAA | 2 | | 1 | 1 | | 4 |

*continued on next page*

**Table 2 (continued)**

Atlantic *Scomber japonicus*. (continued)

| ID | Haplotype | J-FLA | J-ARG | J-SAF | J-IVC | J-ISR | Total |
|---|---|---|---|---|---|---|---|
| 44 | AAGAAAADADAA | | | | 1 | 1 | 2 |
| 45 | AAAAAAADAEAA | | 1 | | | | 1 |
| 46 | AAAAAEADAAAA | | | 1 | | | 1 |
| 47 | AAAAAGGDAAAA | | | | | 1 | 1 |
| 48 | AABAACADAAAA | 1 | | | | | 1 |
| 49 | AACBABABAAAA | 1 | | | | | 1 |
| 50 | AAGAAAADBAAA | | | | 1 | | 1 |
| 51 | AAGAAAAEAAAA | | | 1 | | | 1 |
| 52 | AAGAAAAEABAA | | | 1 | | | 1 |
| 53 | AAGAABADAAAA | | | 1 | | | 1 |
| 54 | AAHAAAADAAAA | | | 1 | | | 1 |
| 55 | ABAAAAADAAAA | | | | | 1 | 1 |
| 56 | ABAAAAADAAAB | | | 1 | | | 1 |
| 57 | ABAAAAADBAAA | | | | 1 | | 1 |
| 58 | BAAAAAADAAAA | | | | | 1 | 1 |

Red Sea *Scomber australasicus*

| ID | Haplotype | A-RED | Total |
|---|---|---|---|
| 59 | BADAAAADDEAA | 2 | 2 |
| 60 | BADAAAADDFAA | 2 | 2 |
| 61 | BADAFAADAFAA | 2 | 2 |
| 62 | BADBAAAAAFAA | 2 | 2 |
| 63 | BADBAAADAFAA | 2 | 2 |
| 64 | AADBAAADAFAA | 1 | 1 |
| 65 | BADAAAADAEAA | 1 | 1 |
| 66 | BADBABADAFAA | 1 | 1 |
| 67 | BALBAAADAFAA | 1 | 1 |
| 68 | BALBAJADAFAA | 1 | 1 |

Unique lineage *Scomber australasicus*

| ID | Haplotype | A-NZL | A-AUS | Total |
|---|---|---|---|---|
| 69 | BCDBBAADABAA | 8 | 6 | 14 |
| 70 | BCDBBAADAAAA | | 1 | 1 |
| 71 | BCDBBAEDABAA | | 1 | 1 |
| 72 | BCDBBAFDABAA | | 1 | 1 |
| 73 | BCDBBBADABAA | 1 | | 1 |
| 74 | BCDBBGADABAA | 1 | | 1 |
| 75 | BCDBBHADABAA | | 1 | 1 |
| 76 | BCDBCAADABAA | 1 | | 1 |
| 77 | BDDBBAADABAA | | 1 | 1 |
| 78 | BDDBBAADABBA | | 1 | 1 |

Ubiquitous lineage *Scomber australasicus*

| ID | Haplotype | A-NZL | A-AUS | A-MEX | A-JPN | Total |
|---|---|---|---|---|---|---|
| 79 | BAEABACFABAA | 1 | 1 | 12 | 9 | 23 |
| 80 | BAEABACEACAA | 6 | 4 | | | 10 |
| 81 | BBEABACFABAA | | | 2 | 1 | 3 |
| 82 | BAEABACFABAA | | | 5 | | 5 |
| 83 | BAKABACFABAA | | | | 3 | 3 |
| 84 | BAEABJCFABAA | | | | 2 | 2 |

*continued on next page*

**Table 2 (continued)**

Ubiquitous lineage *Scomber australasicus* (continued)

| ID | Haplotype | A-NZL | A-AUS | A-MEX | A-JPN | Total |
|---|---|---|---|---|---|---|
| 85 | BAEABABFABAA | | | 1 | | 1 |
| 86 | BAEABACFAFAA | | | | 1 | 1 |
| 87 | BAEABSCFABAA | | | | 1 | 1 |
| 88 | BAEBBACFABAA | | | | 1 | 1 |
| 89 | BAFABACEACAA | 1 | | | | 1 |
| 90 | BBDABACFABAA | | 1 | | | 1 |
| 91 | BBEBBRCFABAA | | | | 1 | 1 |
| 92 | BEKBBACFABAA | | | | 1 | 1 |
| 93 | CAEABACFABAA | | | | 1 | 1 |

*Scomber scombrus*

| ID | Haplotype | S-ENG | S-MAS | Total |
|---|---|---|---|---|
| 94 | ZZZZZZZZZFZZ | 17 | 7 | 24 |
| 95 | ZZZZYZZZZFZZ | | 4 | 4 |
| 96 | ZZZZYZZZZFZZ | | 2 | 2 |
| 97 | CZYZZXZZZFZZ | | 1 | 1 |
| 98 | XZZZZZZZZFYZ | | 1 | 1 |
| 99 | ZXZZZZZZZFZZ | 1 | | 1 |
| 100 | ZYZZYZZZZFZZ | | 1 | 1 |
| 101 | ZZZZXZZZZFZZ | | 1 | 1 |
| 102 | ZZZZZVYZZFZZ | 1 | | 1 |
| 103 | ZZZZZWZZZFZZ | | 1 | 1 |
| 104 | ZZZZZZZXZFZZ | 1 | | 1 |
| 105 | ZZZZZZZYZFZZ | | 1 | 1 |
| 106 | ZZZZZZZZZYZZ | | 1 | 1 |

restriction site data. Only one *S. scombrus* haplotype (no. 94) was shared between the eastern and western North Atlantic samples, and it occurred at significantly different frequencies in each (0.35 in S-ENG, and 0.85 in S-MAS, Table 2). The distribution of haplotypes between the two samples was highly heterogeneous ($P<0.001$), although the estimate of net nucleotide sequence divergence between S-ENG and S-MAS was low ($\delta=0.011\%$), reflecting the close relationship among haplotypes (Fig. 2).

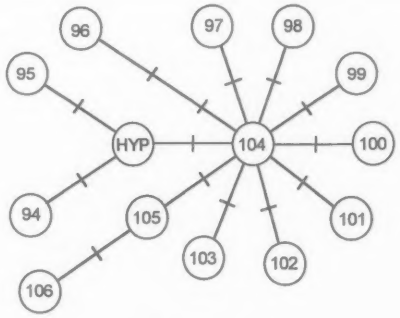



**Figure 2**

Parsimony network illustrating the relationship among mtDNA haplotypes of *Scomber scombrus*. One hypothetical haplotype (HYP) is indicated. Each dash corresponds to a single restriction-site gain or loss.

***Scomber australasicus*** Restriction site analysis of *S. australasicus* mtDNA exhibited a range of intraspecific divergences. Samples collected from Australia and New Zealand were very similar. Three haplotypes (nos. 69, 79, and 80), representing two genetically divergent matrilines, occurred at similar frequencies in each sample (Table 2), and no heterogeneity was revealed ($P=0.718$, $\delta=-0.019\%$). The two samples of *S. australasicus* from the North Pacific (A-JPN and A-MEX) revealed greater divergence, comprising twelve haplotypes, of which two were shared, and one (no. 79) occurred in a large number of individuals in both samples ($n=9$ and $n=12$, respectively). Haplotype 82 occurred in five individuals from A-MEX but was not present in the A-JPN

**Table 3**

Probabilities of significance from Roff and Bentzen's (1989) chi-square analysis with 1000 randomizations of the data of samples of Atlantic *Scomber japonicus*, Pacific *S. japonicus*, *S. australasicus*, and *S. scombrus* that shared haplotypes.

| Comparison | $\chi^2$ | Number of simulations exceeding $\chi^2$ | Probability |
|---|---|---|---|
| Atlantic *Scomber japonicus* | | | |
| J-ISR J-IVC J-SAF J-FLA J-ARG | 191.36 | 0 | <0.001** |
| J-ISR J-IVC J-SAF | 48.70 | 451 | 0.451NS |
| J-ARG J-IVC J-SAF | 60.84 | 28 | 0.028* |
| J-ARG J-IVC | 22.21 | 68 | 0.070NS |
| J-ARG J-SAF | 26.89 | 0 | 0.001** |
| J-FLA J-ARG | 28.37 | 1 | 0.001** |
| J-FLA J-ISR J-IVC | 58.40 | 16 | 0.016* |
| J-FLA J-ISR | 32.00 | 1 | 0.001** |
| J-FLA J-IVC | 24.33 | 14 | 0.014* |
| Pacific *Scomber japonicus* | | | |
| J-JPN J-TWN | 10.67 | 658 | 0.658NS |
| *Scomber australasicus* | | | |
| A-JPN A-MEX A-AUS A-NZL | 128.94 | 0 | <0.001** |
| A-JPN A-MEX | 17.75 | 13 | 0.013* |
| A-AUS A-NZL | 11.67 | 718 | 0.718NS |
| *Scomber scombrus* | | | |
| S-ENG S-MAS | 20.17 | 0 | <0.001** |

*Significantly different at $\alpha$=0.05.
**Significantly heterogeneous at $\alpha$=0.01.
NSNot significant.

***Scomber japonicus*** Of the three species, samples of *S. japonicus* spanned the greatest geographical area, and mtDNA restriction site analysis of this species revealed the largest range of divergences. In the North Pacific, samples from Japan (J-JPN) and Taiwan (J-TWN) shared four haplotypes, three of which occurred in more than one individual in each sample (nos. 1–3, Table 2), and no significant heterogeneity was observed between the samples ($P$=0.658, $\alpha$=0.05, $\delta$=–0.010%). In contrast, a comparison of the pooled data of J-JPN and J-TWN with *S. japonicus* from the eastern North Pacific (J-CAL) revealed one fixed restriction site difference, a net nucleotide sequence divergence of $\delta$=0.30%, and genotype distributions were significantly different ($P$<0.001, $\alpha$=0.05).

Many closely related haplotypes were observed in samples of *S. japonicus* from the Atlantic and Mediterranean Sea, and several were shared among two or more sample locations (Table 2). No significant differences were observed in the distribution of haplotypes among J-ISR, J-IVC, and J-SAF, from the eastern Atlantic ($P$=0.451, $\alpha$=0.05, $\delta$=–0.003%–0.015%, Table 4), or between J-IVC and J-ARG across the Atlantic ($P$=0.07, $\alpha$=0.05, $\delta$=0.025%). All other tests of haplotype frequencies among Atlantic samples were significant ($P$<0.05).

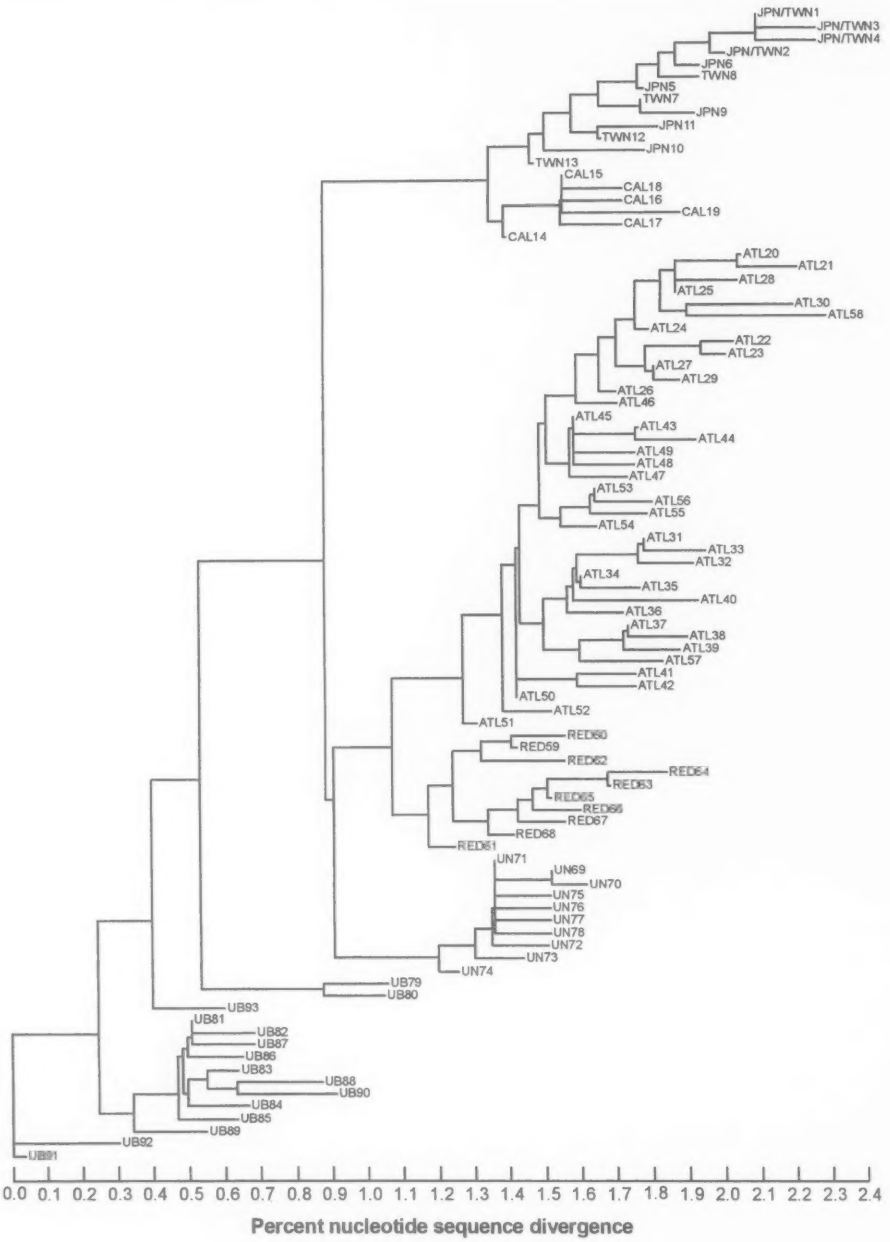
sample. Although the net nucleotide sequence divergence between A-JPN and A-MEX was small ($\delta$=0.021%), the distribution of haplotypes between the samples was significantly heterogeneous ($P$=0.013, $\alpha$=0.05). Together, the Australian and New Zealand samples of *S. australasicus* from the South Pacific were genetically distinct from the combined North Pacific samples (A-JPN and A-MEX). The mean corrected nucleotide sequence divergence between the pooled groups was high ($\delta$=0.54%), and only one haplotype was shared between the pooled South and North Pacific samples (no. 79), occurring at very different frequencies (0.05 and 0.51, respectively).

The Red Sea sample of *S. australasicus* was distinct from all other collections of this species. The mean net nucleotide sequence divergence between A-RED and the pooled data of all other *S. australasicus* was $\delta$=0.86%. The Red Sea sample was less divergent from the *S. australasicus* samples from Australia and New Zealand ($\delta$=0.51%) than were the *S. australasicus* samples from Japan and Mexico ($\delta$=1.2%)

## Phylogeographic patterns

MtDNA restriction site analysis and neighbor-joining of *S. japonicus* and *S. australasicus* mtDNA haplotypes revealed five major clusters: *S. japonicus* from the Pacific Ocean, *S. japonicus* from the Atlantic Ocean, *S. australasicus* from the Red Sea, a "ubiquitous" cluster of haplotypes of *S. australasicus* from all other samples of this species, and a "unique" cluster of only New Zealand and Australia *S. australasicus* haplotypes (Fig. 3).

Parsimony analyses were used to explore cladistic relationships among the haplotypes of *S. japonicus* and *S. australasicus* identified by mtDNA restriction site analysis. Parsimony analysis among all *Scomber* haplotypes could not be conducted because *S. scombrus* mtDNA sequences were so divergent that it was not possible to determine homologous restriction site characters among the three species. Mainly interested in patterns occurring in *S. japonicus*, we chose a root in *S. australasicus* (haplotype no. 92) on the basis of results of parsimony analyses of cytochrome *b* sequences that included *S. scombrus* and a




## Figure 3

Neighbor-joining analysis of mtDNA haplotypes of *Scomber japonicus* and *S. australasicus* identified by restriction site analysis. Branch lengths are indicated. The terminal nodes labeled ATL are Atlantic haplotypes of *S. japonicus* 20–58 from J-FLA, J-ARG, J-SAF, J-IVC, and J-ISR. Haplotypes 1–13 labeled JPN or TWN are *S. japonicus* from J-JPN and J-TWN. Haplotypes 14–9 labeled CAL are *S. japonicus* from J-CAL. Haplotypes 61–68 labeled RED are *S. australasicus* haplotypes from A-RED. Those labeled UN are "unique" haplotypes 69–78 of *S. australasicus* from A-NZL and A-AUS. Those labeled UB are "ubiquitous" haplotypes 79–93 of *S. australasicus* from A-NZL, A-AUS A-MEX, and A-JPN.

more distant outgroup, *Rastrelliger kanagurta*, and a preliminary parsimony tree drawing. Analysis of 58 informative characters yielded 1031 equally parsimonious trees. Each of these 1031 trees shared the same topology among the five major matrilines and differed only within groups, with most rearrangements occurring among Atlantic *S. japonicus* haplotypes. Branches defining the five matrilines were supported at 100% in a majority-rule consensus that illustrated the relationship of all haplotypes of *S. japonicus* and *S. australasicus* and revealed a topology that was not different from that obtained by neighbor-joining (Fig. 4).

The Red Sea sample had haplotypes intermediate to *S. japonicus* and *S. australasicus*, highlighted by character state reversals. Two reversals occurred among the Red Sea *S. australasicus* and the Japan-Taiwan *S. japonicus* clades, and the unique clade of *S. australasicus*. An *Hpa*I site that is absent in eight of the 10 Red Sea haplotypes (nos. 60, 62–68), nine of the 10 unique *S. australasicus* haplotypes (nos. 69–73, 75–78), and all of the *S. japonicus* haplotypes from Japan and Taiwan, is present in all other haplotypes. A *Pvu*II site that is absent in six of the 10 Red Sea haplotypes (nos. 63–68) and all haplotypes of the unique *S. australasicus* clade is present in all other haplotypes. Consequently, corrected nucleotide sequence divergences among the Red Sea and unique *S. australasicus* haplotypes are lower than those among Red Sea and Atlantic *S. japonicus* haplotypes (Table 4). Although UPGMA cluster analysis grouped the Red Sea haplotypes with the unique *S. australasicus* haplotypes (data not shown), neighbor-joining resulted in the placement of the Red Sea sample with Atlantic *S. japonicus*, concordant with the majority-rule consensus. Neighbor-joining is favored over UPGMA because it removes the assumption that the data are ultrametric (the condition that all the taxa are equidistant from the root) (Swofford and Olsen, 1990).

Parsimony analysis of the cytochrome *b* sequences (Table 5) yielded a tree topology that was similar to the patterns observed by parsimony and neighbor-joining of mtDNA restriction site data of *S. japonicus* and *S. australasicus*. However, the analysis showed greater similarity between the unique and ubiquitous *S. australasicus* clades that were not separated by Pacific *S. japonicus*. A majority-rule consensus of two equally parsimonious trees of 86 informative characters (of 93 variable positions) of cytochrome *b* sequences showed that *S. scombrus* is the sister group to *S. japonicus* and *S. australasicus* (Fig. 5). Sequence divergences among the cytochrome *b* sequences were 1.7–3.1% in *S. australasicus*, 0.8–4.3% in *S. japonicus*, and 2.8% in *S. scombrus*. The cytochrome *b* divergences were 2.8–4.0% between haplotypes of *S. japonicus* and *S. australasicus*, and ranged from 14.6 to 17.6% between the *S. scombrus* and the *S. japonicus-S. australasicus* group. The divergence of cytochrome *b* haplotypes between *Rastrelliger kanagurta* and *S. scombrus* was 21.5% and between *Rastrelliger kanagurta* and the *S. japonicus-S. australasicus* group was 17.1–18.8%. Neighbor-joining analysis of divergences among the cytochrome *b* sequences revealed a tree that had equal topology to that in Fig. 5 (data not shown). Parsimony and neighbor-joining of mtDNA restriction site data showed a paraphyletic pattern in both *S. japonicus* and *S.*

**Table 4**

Percent net nucleotide sequence divergences (δ) among samples of *Scomber australasicus* and *Scomber japonicus* derived from mtDNA restriction site data.

| | J-FLA | J-ARG | J-ISR | J-IVC | J-SAF | J-TWN | J-JPN | J-CAL | A-RED | A-AUS | A-NZL | A-JPN | A-MEX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J-FLA | — | | | | | | | | | | | | |
| J-ARG | 0.042 | — | | | | | | | | | | | |
| J-ISR | 0.137 | 0.071 | — | | | | | | | | | | |
| J-IVC | 0.078 | 0.025 | –0.003 | — | | | | | | | | | |
| J-SAF | 0.165 | 0.099 | 0.007 | 0.015 | — | | | | | | | | |
| J-TWN | 1.554 | 1.472 | 1.493 | 1.522 | 1.483 | — | | | | | | | |
| J-JPN | 1.576 | 1.497 | 1.515 | 1.544 | 1.503 | –0.010 | — | | | | | | |
| J-CAL | 1.209 | 1.128 | 1.151 | 1.179 | 1.176 | 0.284 | 0.319 | — | | | | | |
| A-RED | 0.752 | 0.706 | 0.724 | 0.728 | 0.723 | 1.136 | 1.159 | 1.077 | — | | | | |
| A-AUS | 0.934 | 0.907 | 0.874 | 0.892 | 0.861 | 0.974 | 0.990 | 0.886 | 0.489 | — | | | |
| A-NZL | 0.926 | 0.909 | 0.878 | 0.894 | 0.860 | 0.991 | 1.004 | 0.872 | 0.528 | –0.019 | — | | |
| A-JPN | 1.640 | 1.599 | 1.560 | 1.579 | 1.505 | 1.523 | 1.529 | 1.533 | 1.176 | 0.554 | 0.477 | — | |
| A-MEX | 1.688 | 1.654 | 1.604 | 1.622 | 1.543 | 1.602 | 1.606 | 1.607 | 1.267 | 0.619 | 0.533 | 0.021 | — |

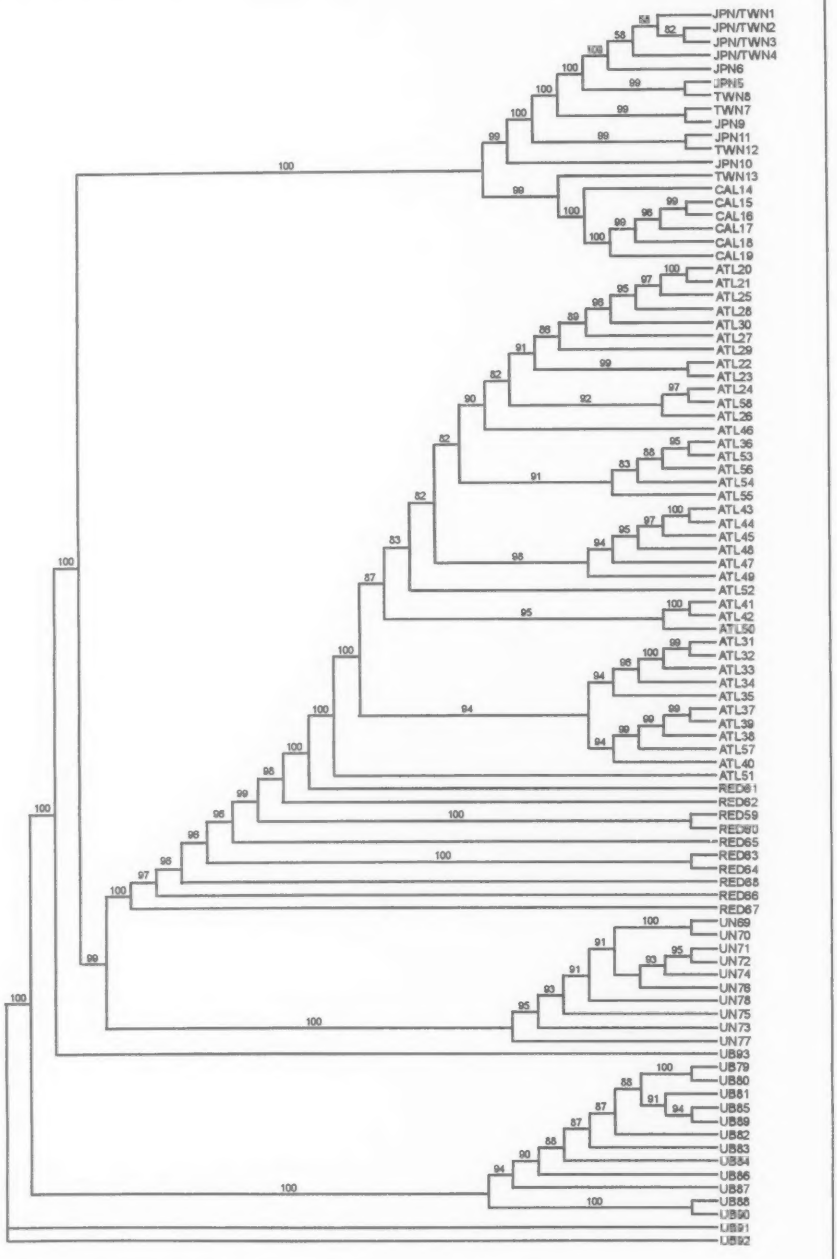



**Figure 4**

Majority rule consensus of 1031 equally parsimonious trees of *Scomber japonicus* and *S. australasicus* mtDNA haplotypes identified by restriction site analysis. Each of the 1031 equally parsimonious trees has the same topology among the major clades, with rearrangements only within groups. Numbers at nodes indicate the percentage of times the node was supported in 1000 bootstrap replications with a heuristic search. There were 58 informative characters used to draw the tree which is 118 steps in length. The consistency index is 0.86. Terminal nodes are labeled as in Figure 3.

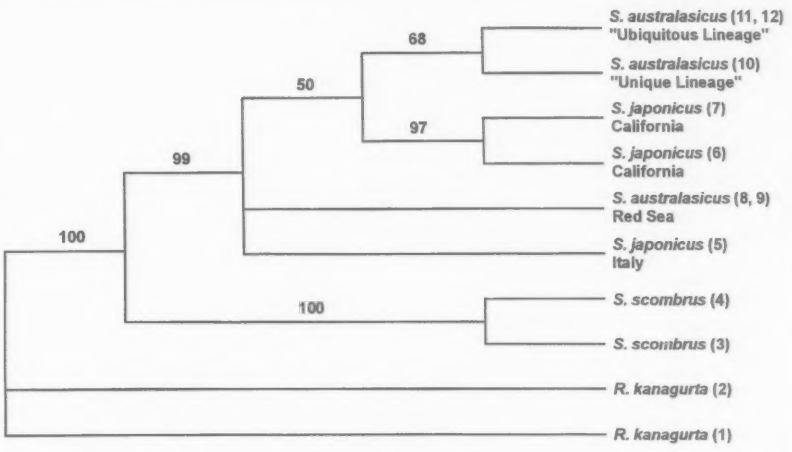



**Figure 5**

Majority rule consensus of two equally parsimonious trees drawn by using cytochrome *b* sequences of selected individuals of the major lineages chosen on the basis of results of parsimony and cluster analyses of restriction site data. The sequence numbers in parentheses correspond to the numbered sequences in Table 5. The tree is rooted to *Rastrelliger kanagurta* (sequence no. 2). Numbers at nodes indicate the percentage of times the node was supported in 1000 bootstrap replications with a heuristic search. There were 86 informative characters used to draw the tree which is 113 steps in length. The consistency index is 0.89.

*australasicus*. Parsimony analysis of cytochrome *b* sequences also revealed paraphyly in both *S. japonicus* and *S. australasicus*.

## Discussion

### Relationships at the generic level

Early studies of *Scomber* demonstrated considerable morphological divergence between species. Characterized by the presence of a swimbladder, *S. japonicus* and *S. australasicus* were once placed in the genus *Pneumatophorus*, leaving only *S. scombrus* in the genus *Scomber* (Starks, 1921). The two genera were subsequently united because many other morphological characters were shared among the three species (Fraser-Brunner, 1950; Matsui, 1967) and because the presence of a swimbladder can vary intraspecifically in the Scombridae (Collette and Gibbs, 1963).

Concordant with morphological dissimilarities between these two groups, the level of genetic divergence between *S. scombrus* and the *S. australasicus-S. japonicus* group estimated from mtDNA restriction fragment data was high (δ=11.2%). The divergence of these groups estimated from direct sequence analysis of the cytochrome *b* gene was also high (δ=16.7%). For comparison, we calculated divergences among 29 scombroid species and drew a phenetic tree (UPGMA), using the 600-bp cytochrome *b* sequences reported by Block et al. (1993).[2] The lowest intergeneric divergence in the tree was δ=3.0% between *Makaira* and *Istiophorus*, whereas the greatest was δ=31% between *Trichiurus* and *Gempylus*. The greatest divergence between species within a single genus was δ=14.6% between *Scomber japonicus* and *S. scombrus*, slightly lower than our estimate. Other interspecific divergences were δ=13.5% between *Scomberomorus cavalla* and *Scomberomorus maculatus*, 3.9% between *Sarda chiliensis* and *Sarda sarda*, 1.2–5% among five species of *Thunnus*, and 0.3–5.2% among five species of *Tetrapturus*. Although the clonal inheritance of mtDNA can result in high levels of divergence compared with estimates determined from nuclear DNA markers, the high values seen in *Scomber* suggest that a re-evaluation of generic taxonomy in *Scomber* may be in order.

### Relationships at the species level

The current taxonomy of *Scomber* is based on multiple morphological characters, including the number of interneural bones under the first dorsal fin, length of the space between the dorsal fins in rela-

[2] GenBank accession nos. L11532-L11562.

## Table 5

DNA sequences of cytochrome *b* of 12 individuals of *Scomber*, and *Rastrelliger kanagurta*, with reading frame indicated. Reported sequences of *S. japonicus* from Italy[1] and *S. scombrus*, sequences nos. 3 and 5, are from Finnerty and Block (1992). Indicated positions are relative to the full-length human mitochondrial DNA sequence[2] (Anderson et al., 1981). A '.' indicates identity to sequence number 1, and a '–' represents missing data.

| | Species | GenBank | Haplotype | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | 15085 | | | | 15100 |
| 1 | *R. kanagurta* | AF032704 | Unknown | | ta | gaa | aca | tga | aac | atc | gga | gtt | g-- | | | |
| 2 | *R. kanagurta* | AF032705 | Unknown | | | | | | | | | | | | | |
| 3 | *S. scombrus* | L11544 | Unknown | t | g.. | ..g | ... | ... | ... | g.. | ..t | ..a | .tc | | | |
| 4 | *S. scombrus* | AF032706 | S-MAS94 | | .. | ..g | ... | ... | ... | g.. | ..t | ..a | .tc | | | |
| 5 | *S. japonicus* (Italy) | L11546 | Unknown | t | a.g | ..g | ... | ... | ... | ..t | ..t | ..g | .tt | | | |
| 6 | *S. japonicus* | AF032707 | J-CAL15 | c | a.g | ... | ... | ... | ... | ..t | ..t | ..g | .tt | | | |
| 7 | *S. japonicus* | AF032708 | J-CAL14 | | a.g | ... | ... | ... | ... | ..t | ..t | ..g | .tt | | | |
| 8 | *S. australasicus* | AF032709 | A-RED62 | | .g | ..- | ... | ... | ... | ..t | --- | --- | --- | | | |
| 9 | *S. australasicus* | AF032710 | A-RED60 | | | | | | | | gt | ..a | .tt | | | |
| 10 | *S. australasicus* | AF032711 | A-AUS71 | c | a.g | ... | ... | ... | ... | ..t | ..t | ..a | .tt | | | |
| 11 | *S. australasicus* | AF032712 | A-MEX79 | | | | | | | | ..t | ..a | .tt | | | |
| 12 | *S. australasicus* | AF032712 | A-MEX81 | | | | | | | | ..t | ..a | .tt | | | |

| | 15115 | | | | | | | 15130 | | | | | | 15145 | | | | | | 15160 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ctt | ctc | ctc | tta | gta | atg | ata | acc | gct | ttc | gtt | ggc | tac | gtc | ctt | ccc | tga | gga | caa | atg |
| 2 | .. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ..c | ... | ... | c.c | ... | ... | ..g | ..t | ..c | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | ..c | ... | ... | c.c | ... | ... | ..g | ..t | ..c | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..t | ... | ... |
| 6 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..c | ... | ... |
| 7 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..c | ... | ... |
| 8 | --- | --- | --- | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..t | ... | ... |
| 9 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..t | ... | ... |
| 10 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..c | ... | ... |
| 11 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..c | ... | ..a |
| 12 | ... | ... | ... | c.c | ... | ..a | ... | ... | ..a | ... | ..c | ... | ... | ... | ... | ... | ... | ..c | ... | ..a |

| | 15175 | | | | | | | 15190 | | | | | | 15205 | | | | | | 15220 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | tcc | ttc | tga | ggt | gca | act | gtc | att | act | aac | ctc | ctt | tcc | gca | gtc | cct | tat | gta | ggc | act |
| 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ... | ... | ... | ..a | ..c | ... | ... | ..c | ... | ... | ..a | ..c | ..a | ... | ..t | ..a | ..c | ... | ... | ..a |
| 4 | ... | ... | ... | ..g | ..c | ... | ... | ..c | ... | ... | ..a | ..c | ..a | ... | ..t | ..a | ..c | ... | ... | ..a |
| 5 | ... | ... | ... | ..g | ..c | ... | ... | ... | ..g | ... | ..a | ..c | ..g | ... | ... | ..a | ..c | ..t | ..t | ... |
| 6 | ... | ... | ... | ..g | ..c | ..c | ... | ... | ..a | ..t | ..a | ... | ..a | ... | ... | ..a | ..c | ..c | ..t | ... |
| 7 | ... | ... | ... | ..g | ..c | ..c | ... | ... | ..a | ..t | ..a | ... | ..g | ... | ... | ..a | ..c | ..c | ..t | ... |
| 8 | ... | ... | ... | ..a | ..c | ... | ... | ... | ..a | ... | ..a | ... | ..g | ... | ... | ..a | ..c | ..c | ..t | ... |
| 9 | ... | ... | ... | ..a | ..c | ... | ... | ... | ..a | ... | ..a | ... | ..g | ... | ... | ..a | ..c | ..c | ..t | ... |
| 10 | ... | ... | ... | ..g | ..c | ..c | ... | ... | ..a | ... | ..a | ... | ..g | ... | ... | ..a | ..c | ..c | ..t | ... |
| 11 | ... | ... | ... | ..g | ..c | ..c | ... | ... | ..a | ... | ..a | ... | ..g | ... | ... | ..a | ..c | ... | ..t | ... |
| 12 | ... | ... | ... | ..g | ..c | ..c | ... | ... | ..a | ... | ..a | ... | ..g | ... | ... | ..a | ..c | ... | ..t | ... |

| | 15235 | | | | | | | 15250 | | | | | | 15265 | | | | | | 15280 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | acc | cta | gta | gaa | tga | atc | tga | ggt | ggc | ttc | tcc | gtc | gac | aat | gca | acc | ctc | act | cga | ttc |
| 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ..a | ..c | ..t | ... | ... | ..t | ... | ..g | ... | ... | ..a | ..a | ... | ... | ..t | ..g | ... | ... | ..g | ... |
| 4 | ..a | ..c | ..t | ... | ... | ..t | ... | ..g | ... | ... | ..a | ..a | ... | ... | ..t | ..g | ... | ... | ..g | ... |
| 5 | ..a | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 6 | ... | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | t.c | ... | ... | ..c | ... | ... |
| 7 | ... | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 8 | ..a | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 9 | ..a | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 10 | ..a | ..c | ..t | ..g | ... | ... | ..g | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 11 | ..a | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 12 | ..a | ..c | ..t | ..g | ... | ... | ... | ..c | ... | ... | ..a | ..a | ... | ... | ..c | ... | ... | ..c | ... | ... |

*continued on next page*

tion to the dorsal groove length, and relative positions of the anal and dorsal fin origins (Matsui, 1967). Previous genetic data supported the specific status of *S. australasicus* and *S. japonicus*: allozyme electrophoresis revealed fixed allelic differences between *S. australasicus* and Pacific *S. japonicus* and pro-

**Table 5 (continued)**

| | 15295 | | | | | | | | 15310 | | | | | | | 15325 | | | | | 15340 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ttc | gca | ttc | cat | ttc | ctt | tac | ccg | ttc | gtc | atc | gca | gca | ata | aca | atc | ctg | cac | ctt | ctc |
| 2 | ... | ... | ... | ... | ... | ... | .t. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ... | ..c | ..t | ..c | ... | ..a | .t. | ..c | ..t | ..t | ... | tt. | ..g | gc. | g.g | g.t | ..c | ... | ..a | ..a |
| 4 | ... | ..c | ..t | ..c | ... | ..a | .t. | ..c | ..t | ..t | ... | tt. | ..g | gc. | g.g | g.t | ..c | ... | ..a | ..a |
| 5 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ... | ... | ctg | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 6 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ..t | ctg | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 7 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ..t | ctg | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 8 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ... | ctg | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 9 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ... | ctg | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 10 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ... | -t. | ..g | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 11 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ..t | ct. | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |
| 12 | ..t | ..c | ... | ..c | ... | ..a | .t. | ..a | ... | ..t | ..t | ct. | ... | gc. | ... | ..t | ..t | ... | ..g | ..a |

| | 15355 | | | | | | | | 15370 | | | | | | | 15385 | | | | | 15400 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ttc | cta | cat | gaa | act | gga | tca | aag | aac | cca | atg | ggc | cta | aac | tca | aat | gca | gat | aaa | atc |
| 2 | ... | ... | ... | ... | ... | ... | ... | ..c | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ... | ..t | ... | ... | ... | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..t | ... | ... | ..c | ... | ... |
| 4 | ... | ..t | ... | ... | ... | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..t | ... | ... | ..c | ... | ... |
| 5 | ... | ... | ... | ... | ..c | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..t | ... | ... | ..c | ... | ... |
| 6 | ... | ... | ... | ..g | ..c | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..c | ... | ... | ..c | ..g | ... |
| 7 | ... | ... | ... | ..g | ..c | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 8 | ... | ... | ... | ... | ..c | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 9 | ... | ... | ... | ... | ..c | ..g | ... | ..c | ... | ... | ..t | ... | ... | ... | ..c | ... | ... | ..c | ... | ... |
| 10 | ... | ... | ... | ... | ..c | ..g | ..g | ..c | ... | ... | ..t | ... | ... | ... | ..c | ... | ..g | ..c | ... | ... |
| 11 | ... | ... | ... | ... | ..c | ..g | ..g | ..c | ... | ... | ..t | ... | ... | ... | ..t | ... | ..g | ..c | ... | ... |
| 12 | ... | ... | ... | ... | ..c | ..g | ..g | ..c | ... | ... | ..t | ... | ... | ... | ..t | ... | ..g | ..c | ... | ... |

| | 15415 | | | | | | | | 15430 | | | | | | | 15445 | | | | | 15460 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | tcc | ttc | cac | ccc | tac | ttc | acc | tac | aaa | gat | gcc | cta | gga | ttt | gcc | atc | ctt | ctt | atg | gct |
| 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ..g | ... | ..t | ..g | ... | ..t | ..g | ..t | ... | ..c | ct. | ..c | ..c | ... | ... | g.t | ..c | ... | ..a | .gc |
| 4 | ..g | ... | ..t | ..g | ... | ..t | ..g | ..t | ... | ..c | ct. | ..c | ..c | ... | ... | g.t | ..c | ... | ..a | .gc |
| 5 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ..c | ..c | g.a | ..c |
| 6 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ..c | ..c | g.. | ..c |
| 7 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ..c | ..c | g.. | ..c |
| 8 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ..c | ..c | g.. | . |
| 9 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ..c | ..c | g.. | ..c |
| 10 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ... | ..c | g.. | ..c |
| 11 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ... | ..c | g.. | ..c |
| 12 | ... | ... | ... | ..a | ... | ... | ... | ... | ... | ..c | ct. | ..t | ..c | ... | ... | g.. | ... | ..c | g.. | ..c |

| | 15475 | | | | | | | 15490 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ctt | aaa | tcc | cta | gca | ctc | ttc | tcc | ccc | aac | ct |
| 2 | .. | | | | | | | | | | |
| 3 | ... | .cc | ... | ... | ... | ... | ... | ... | ..g | ... | . |
| 4 | ... | .cc | ... | ... | ... | ... | ... | ... | ..g | ... | . |
| 5 | ... | tcc | ..t | ... | ... | ... | . | | | | |
| 6 | ..c | tcc | ..t | ..c | ... | ... | ... | ..t | ... | ... | .. |
| 7 | ..c | tcc | ..t | ..c | ... | ... | ... | ..t | ... | ... | .. |
| 8 | | | | | | | | | | | |
| 9 | ... | tcc | .. | | | | | | | | |
| 10 | ..c | tcc | ..t | ..c | ... | ... | ... | c.t | ... | ... | .. |
| 11 | ..c | tcc | ..t | .. | | | | | | | |
| 12 | ..c | tcc | ..t | .. | | | | | | | |

[1] Block, B. 1993. Personal communication.
[2] GenBank accession no. V00662.

vided no evidence for the hybrid origin of individuals of intermediate coloration (Kijima et al., 1986).

The systematic status of geographically distant *S. japonicus* populations has been problematic because this species is characterized by considerable morphological variability. Several names have been used for the groups occurring in different areas: *S. japonicus*, western North Pacific (Houttuyn, 1782); *S. colias*, eastern Atlantic (Gmelin, 1789); *S. grex*, western North Atlantic (Mitchell, 1815); *S. diego*, eastern North Pacific (Ayres, 1857); *S. peruanus*, eastern South Pacific (Jordan and Hubbs, 1925); and *Pneumatophorus japonicus marplatensis*, western South Atlantic (López, 1955). Differences in pigmentation, tooth crenulation, scale size, and gill-raker counts on the lower first arch distinguish fish from the Pacific, western Atlantic, and eastern Atlantic. These groups were synonymized into the single species *S. japonicus* by Matsui (1967) because "[morphological] differences are smaller [between populations] than those which separate sympatric species of *Scomber* and *Rastrelliger*, and it thus seems correct to regard *S. japonicus* as a single polytypic species."

The mtDNA data did not refute the specific status of *S. japonicus* and *S. australasicus* reported by Matsui (1967). In fact, a greater divergence was found among two mtDNA lineages within *S. australasicus* than between any other pairs of samples of *S. japonicus* and *S. australasicus*. The two divergent *S. australasicus* mtDNA lineages, which differed by an average of δ=1.84% according to the restriction site data, were equally represented in both South Pacific collections. A cursory allozyme analysis of 10 loci from 10 individuals of each lineage revealed no significant allelic differences in nuclear encoded genes (Scoles, 1994). The phylogenetic pattern observed in *S. australasicus* appears to be the result of postdivergence introgressive hybridization.

A similar phylogenetic pattern has been observed in other pelagic marine fishes. In blue marlin (*Makaira nigricans*), "ubiquitous" haplotypes occurring in both the Atlantic and Pacific oceans were highly divergent from other Atlantic haplotypes, forming a "unique" clade revealed by mtDNA analysis restriction fragment analysis ($n$=114, δ=0.15%, Graves and McDowell, 1995) and by sequencing 612 bp of the mtDNA cytochrome *b* gene ($n$=26, δ=1.6%, Finnerty and Block, 1992). Similarly, sailfish (*Istiophorus platypterus*) had Pacific and Atlantic clades that differed by δ=0.27% (Graves and McDowell, 1995). The patterns in these fishes and in *S. australasicus* demonstrate that considerable mtDNA divergence can occur among haplotypes within a single sample, and suggest the limitations of basing taxonomic status only on mtDNA data.

The mtDNA data indicated the possibility of multiple taxonomic lineages within *S. japonicus*. The two major groups of *S. japonicus* (Atlantic and Pacific) are differentiated by nearly as much as are the two different lineages within *S. australasicus*. The nucleotide sequence divergence between some *S. japonicus* groups is nearly as great as between groups of *S. japonicus* and *S. australasicus* (Table 4). Unlike the two divergent *S. australasicus* lineages that were observed in single samples, the *S. japonicus* groups are geographically partitioned. The paraphyletic relationship (Figs. 3–5), geographic isolation, high level of genetic divergence (Table 4), and morphological differences among the *S. japonicus* lineages support recognition of separate species: *Scomber japonicus* Houttuyn, 1782, in the Pacific; and *Scomber colias* Gmelin, 1789, the Atlantic. This conclusion assumes that there is no significant sampling error (see Moore, 1995) and should be tested by further morphological and nuclear DNA data analyses. The Red Sea population of *Scomber*, previously considered as *S. japonicus* (Matsui, 1967), was re-examined by Baker and Collette (1998), who have assigned the Red Sea-northern Indian Ocean population to *Scomber australasicus* on the basis of morphological characters.

## Phylogeographic patterns and their origins

The distribution of haplotypes between eastern and western Atlantic samples of *S. scombrus* indicated the populations do not share a common gene pool. The close relationship among haplotypes in these samples (Fig. 2) contrasts with the finding of two divergent mtDNA matrilines (δ=3.7%) in samples of capelin, *Mallotus villosus*, from eastern and western regions of the North Atlantic (Dodson et al., 1991). An intermediate level of divergence to that of *S. scombrus* and of capelin was observed between samples of bluefish (*Pomatomus saltatrix*, δ=0.26%, 1 fixed difference) from the eastern and western Atlantic Ocean (Goodbred and Graves, 1996). The lower divergence in *S. scombrus* may be the result of more recent isolation or greater vagility.

Patterns of divergence in *S. japonicus* and other species in the Atlantic Ocean appear to be highly influenced by species' vagility and warm water in the tropics. Haplotype distributions were significantly different between *S. japonicus* samples from the western Atlantic Ocean (J-FLA and J-ARG), and unique haplotypes occurred within each at relatively high frequencies (10–20%). Similary, a population-genetic study of the shortfin mako shark demonstrated significantly different mtDNA haplotype frequencies between samples from the western North and South Atlantic Ocean (δ=0.13%, $P$<0.001) (Heist

et al., 1996). Bluefish sampled from Brazil and the eastern U.S. coast were even more divergent (δ=1.48%) (Goodbred and Graves, 1996). But in the eastern Atlantic, no significant differentiation was seen among *S. japonicus* samples from the eastern Mediterranean Sea (J-ISR), Ivory Coast (J-IVC), and South Africa (J-SAF). Additionally, although no haplotypes were shared between bluefish samples from the Mediterranean Sea and South Africa (δ=0.35%), haplotypes in the two samples were closely related (Goodbred and Graves, 1996). In contrast, the less mobile gray mullet, *Mugil cephalis*, sampled from the Mediterranean Sea, and South Africa were highly divergent (δ=2.92) (Crosetti et al., 1994). These data suggest that fewer barriers to gene flow occur between northern and southern regions of the eastern Atlantic versus those of the western Atlantic. The tropical water of the east Atlantic occupies the smallest area of all the tropical regions, and temperate species are suspected of crossing this region by moving under the warm water mass, thereby achieving an antitropical distribution (Briggs, 1974).

It is possible that *S. japonicus* larval or adult exchange occurs between Gulf of Guinea and the northernmost extension of the southwest Atlantic *S. japonicus* population assisted by trans-Atlantic equatorial currents. These locations are separated by the most narrow region of the Atlantic Ocean. Haplotype frequencies were significantly different among all samples across the Atlantic Ocean except in one pairwise comparison of J-ARG and J-IVC. Genetic similarity between these samples may reflect recent isolation or contemporary gene flow. Eastern and western Atlantic populations of *S. japonicus* are, however, morphologically differentiated on the basis of nonoverlapping gill-raker counts (Starks, 1921; Matsui, 1967).

The sample of *S. australasicus* from the Red Sea (A-RED) had the highest estimated haplotypic diversity. No haplotypes were shared between the samples of *S. japonicus* from the Atlantic Ocean and the Red Sea, suggesting no gene flow through the Suez Canal. Fifty-two fish species are known to have made the passage, all but one from the Red Sea to the Mediterranean Sea (Golani, 1993). *Rastrelliger kanagurta*, a close relative of *Scomber*, is among those known to have invaded the Mediterranean Sea from the Red Sea (Collette, 1970). The samples of *S. japonicus* from the eastern Mediterranean Sea (J-ISR) and *S. australasicus* from the Red Sea were divergent by δ=0.72%, one fixed restriction site difference, and an additional site that was nearly fixed. These findings do not exclude the possibility of movement through the Suez Canal, which may be revealed by larger sample sizes.

Fishery data indicate that *S. japonicus* in the region of Japan and Taiwan represent separate populations with unique spawning grounds and larval retention areas (Sato, 1990). Although haplotypes in the sample from the eastern Pacific (J-CAL) were not found in other *S. japonicus* samples, haplotypes in *S. japonicus* samples from the northwest region of the Pacific Ocean (J-JPN and J-TWN) were shared at similar frequencies. The data of our study are consistent with exchange among the two western Pacific populations. Whether exchange is the result of larval drift or adult movement is indeterminable; however, movement of adult *S. australasicus* is known to occur between these regions (Chang and Wu, 1977). Greater trans-Pacific divergence than that observed in *S. japonicus* was seen in gray mullet sampled from Hawaii and the Galapagos Islands (δ=1.4%) (Crosetti et al., 1994), suggesting these population were isolated for a considerably longer period than mackerel.

The level of nucleotide sequence divergence between Atlantic and Pacific samples of *S. japonicus* was lower than expected. It has been suggested that in mammals, mitochondrial DNA evolves at rate of 2% nucleotide sequence divergence per million years (Brown et al., 1979). When this rate of divergence is applied to the 1.4% net nucleotide sequence divergence observed between Atlantic and Pacific *S. japonicus*, it appears that the two populations were isolated about 700,000 years. Such estimates of divergence are approximate at best, but if this rate of divergence is valid in *Scomber japonicus*, the observed nucleotide sequence divergence is lower than would have resulted if these populations were isolated by the uplift of the Isthmus of Panamá, 3.1–3.6 million years ago (Keigwin, 1978). Gene flow may have occurred between the two regions since the uplift of Panamá, during times of warmer climates (Avise, 1992).

Samples of *S. australasicus* between New Zealand and Australia, regions separated by nearly 2000 km of deep ocean, were genetically homogeneous. Perhaps there is a high potential for genetic exchange in *S. australasicus*, which was also indicated by comparison of *S. australasicus* samples from the northern Pacific. Although a test of homogeneity between A-JPN and A-MEX was marginally significant ($P$=0.013), the mean nucleotide sequence divergence was only 0.02%, which contrasted with the 0.30% divergence and fixed restriction site difference between samples of *S. japonicus* from the eastern and western Pacific Ocean.

The lowest genotypic and nucleotide sequence diversity in the study occurred in a sample from the Revillagigedo Islands (A-MEX) and may indicate the origin of this population by means of a founder event

from a source population in the northwest Pacific region or Hawaii. Colonization of the Revillagigedo Islands may have occurred through the migration of adults. Gene flow between other isolated populations (J-IVC and J-ARG; A-NZL and A-AUS) was also suggested by the mtDNA data and may be facilitated by the high vagility of species of *Scomber*.

Despite the genetic similarity observed among widely separated samples of *S. australasicus*, exchange in the north-south direction is restricted. Haplotypes of the unique New Zealand-Australia lineage were not observed in samples from Japan or Mexico. These data suggest that the equatorial region of the Pacific is a barrier to gene flow in *S. australasicus*, and together with the significant difference observed between western Atlantic *S. japonicus* samples, strongly emphasizes the impact of physical oceanographic conditions in shaping phylogeographic patterns. *Scomber japonicus* from the eastern North and South Pacific were also differentiated by allozyme analysis which revealed three differentiated loci of fourteen that were variable (Nei's $D$=0.033, $F_{st}$=0.20) (Stepien and Rosenblatt, 1996). These observations support the hypothesis that physical oceanographic characteristics of equatorial regions may limit gene flow in species of *Scomber*, and possibly a variety of other temperate pelagic species.

These data demonstrate that levels of heterogeneity reflect species vagilities and are shaped by physical oceanographic processes. Other studies have demonstrated that population structure in several species is generally lower between populations separated by vast ocean basins than between those separated by land masses, in some cases suggesting continued gene flow (Vawter et al., 1980; Rosenblatt and Waples, 1986; Shulman and Bermingham, 1995). But not all genetic data support a simple relationship between the degree of geographic barriers, dispersal, and genetic relatedness. In one study, five species with various life history features that limit dispersal showed no intraspecific differentiation, whereas one, *Gnatholepis thompsoni*, which had nonpelagic eggs and a long planktonic life, showed population structure across the Caribbean Sea (Shulman and Bermingham, 1995). Sinclair (1988) reviewed the effects of oceanographic characteristics on population structure and concluded that it is defined at the planktonic phase of the life history, rather than at the juvenile or adult phases, and is largely regulated by the physical environment. However, Thresher and Brothers (1985) were unable to relate larval stage duration or adult size to dispersal in several angelfish species (Pomacanthidae) and suggested that geological features are better predictors of dispersal. Clearly, the processes by which population structure emerges in marine fishes are complex. Our data support the hypothesis that oceanographic characteristics are significant in limiting dispersal in *Scomber*, especially in some equatorial regions. The exchange of adult individuals among populations appears to have a significant effect on limiting the divergence between some populations, possibly reflecting adaptations in *Scomber* for survival in the pelagic environment.

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## Literature cited

**Anderson, S., A. T. Bankier, B. G. Barrell, M. H. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. Smith, R. Staden, and I. G. Young.**
**1981.** Sequence and organization of the human mitochondrial genome. Nature (Lond.) 290:457–465.

**Avise, J. C.**
**1992.** Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 62:62–76.

**Ayres, W. O.**
**1857.** Description of a new species of mackerel, *Scomber diego*, Ayres. Proc. Calif. Acad. Sci. 1:92.

**Baker, E., and B. B. Collette.**
**1998.** Mackerel from the northern Indian Ocean and the Red Sea are *Scomber australasicus*, not *Scomber japonicus*. Ichthyol. Res. 45(1):29–33.

**Block, B. A., J. R. Finnerty, A. F. R. Stewart, and J. Kid**
**1993.** Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. Science (Wash., D.C.) 260:210–214.

**Briggs, J. C.**
**1974.** Marine zoogeography. McGraw-Hill Series in Population Biology. McGraw-Hill. New York, NY, 475 p.

**Brown, W. M., M. George Jr., and A. C. Wilson.**
**1979.** Rapid evolution of animal mitochondrial DNA. Proc. Nat. Acad. Sci. USA 76:1967–1971.

**Chang, K. -H., and W. -L. Wu.**
**1977.** Tagging experiments on the spotted mackerel (*Scomber australasicus*) in Taiwan. Bull. Inst. Zool., Academia Sinica 16:137–139.

**Chapman, R. W., and D. A. Powers.**
**1984.** A method for the rapid isolation of mitochondrial DNA from fishes. Maryland Sea Grant College Program Tech. Rep. UM-SG-TS-84-01, 11 p.

**Collette, B. B.**
**1970.** *Rastrelliger kanagurta*, another Red Sea immigrant into the Mediterranean Sea, with a key to the Mediterranean species of Scombridae. Bull. Sea Fish. Res. Stn. (Haifa) 54:3–6.

**Collette, B. B., and R. H. Gibbs Jr.**
**1963.** A preliminary review of the fishes of the family Scombridae. Proc. World Sci. Meet. Biol. Tunas, FAO Fish. Rep. no. 6, 1:23–32.

**Collette, B. B., and C. E. Nauen.**
**1983.** FAO species catalogue. Vol. 2: Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO Fish. Synop. 125, 137 p.

**Collette, B. B., T. Potthoff, W. J. Richards, S. Ueyanagi, J. L. Russo, and Y. Nishikawa.**
**1984.** Scombroidei: development and relationships. *In* H. G. Moser et al. (eds.), Ontogeny and systematics of fishes, p. 591–620. Am. Soc. Ichthyol. Herpetol. Spec. Publ. 1.

**Crosetti, D., W. S. Nelson, and J. C. Avise.**
**1994.** Pronounced genetic structure of mitochondrial DNA among populations of the circumglobally distributed grey mullet (*Mugil cephalus*). J. Fish Biol. 44:47–58.

**Dodson, J. J., J. E. Carscadden, L. Bernatchez, and F. Colombani.**
**1991.** Relationship between spawning mode and phylogeographic structure in mitochondrial DNA of North Atlantic capelin *Mallotus villosus*. Mar. Ecol. Prog. Ser. 76:103–113.

**Finnerty, J. R., and B. A. Block.**
**1992.** Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Mol. Mar. Biol. Biotechnolog. 1:206–214.

**Fraser-Brunner, A.**
**1950.** On the fishes of the family Scombridae. Ann. Mag. Nat Hist. Ser. 12, 3:131–163.

**Gmelin, J. F.**
**1789.** Caroli a Linné...systema naturae, vol. 1, pt. 3, Pisces Lipsiae, p. 1126–516.

**Golani, D.**
**1993.** The biology of the Red Sea migrant, *Saurida undosquamis* in the Mediterranean and comparison with the indigenous confamilial *Synodus saurus* (Teleostei: Synodontidae). Hydrobiologia 271:109–117.

**Goodbred, C. O., and J. E. Graves.**
**1996.** Genetic relationships among geographically isolated populations of bluefish (*Pomatomus saltatrix*). Mar. Freshwater Res. 47:347–355.

**Graves, J. E., and A. E. Dizon.**
**1989.** Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). Can. J. Fish. Aquat. Sci. 46:870–873.

**Graves, J. E., S. D. Ferris, and A. E. Dizon.**
**1984.** Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Mar. Biol. 79:315–319.

**Graves, J. E., and J. R. McDowell.**
**1995.** Inter-ocean genetic divergence of istiophorid billfishes. Mar. Biol. 122:193–203.

**Hamre, J.**
**1980.** Biology, exploitation and management of the Northeast Atlantic mackerel. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 177:212–242.

**Heist, E. J., J. A. Musick, and J. E. Graves.**
**1996.** Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. Can. J. Fish. Aquat. Sci. 53:583–588.

**Houttuyn, M.**
**1782.** Beschryving van eenige Japansce visschen, en andere zee-schepzelen. Verh. Holl. Maatsch. Wet. Haarlem 20(2):311–350.

**Hunter, J. R., and C. A. Kimbrell.**
**1980.** Early life history of Pacific mackerel, *Scomber japonicus*. Fish. Bull. 78:89–101.

**Jamieson, A., and P. J. Smith.**
**1987.** Atlantic mackerel (*Scomber scombrus* L.) stocks and genes: a review. J. Cons. Int. Explor. Mer 44:66–72.

**Jordan, D. S., and C. L. Hubbs.**
**1925.** Record of fishes obtained by David Starr Jordan in Japan, 1922. Mem. Carnegie Mus. 10(2):93–346.

**Keigwin, L. D.**
**1978.** Pliocene closing of the isthmus of Panamá, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. Geology 6:630–634.

**Kijima, A., N. Taniguchi, and A. Ochiai.**
**1986.** Genetic divergence and morphological difference between the spotted and common mackerel. Japan. J. Ichthyol. 33:152–161.

**Lacson, J. M.**
**1992.** Minimal genetic variation among samples of six species of coral reef fishes collected at La Parguera, Puerto Rico, and Discovery Bay, Jamaica. Mar. Biol. 112:327–331.

**López R. B.**
**1955.** La caballa del mar Argentino. Datos sobre su biología y pesca. Rev. de la Asoc. Arg. Dietologia 13:25–33.

**MacKay, K. T., and E. T. Garside.**
**1969.** Meristic analysis of Atlantic mackerel, *Scomber scombrus*, from the North American coastal population. J. Fish. Res. Board Can. 26:2537–2540.

**Maguire, J. -J., Y. C. Chagnon, M. Castonguay, and B. Mercille.**
**1987.** A review of mackerel management areas in the Northwest Atlantic. CAFSAC (Canadian Atlantic Fisheries Scientific Advisory Committee) Res. Doc. 87/71, 31 p.

**Matsui, T.**
**1967.** Review of the mackerel genera *Scomber* and *Rastrelliger* with description of a new species of *Rastrelliger*. Copeia 1967:71–83.

**McElroy, D., P. Moran, E. Bermingham, and I. Kornfield.**
**1992.** REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. 83:157–158.

**Mitchell, S. L.**
**1815.** The fishes of New-York, described and arranged. Trans. Lit. Philos. Soc. N.Y. 1:355–492.

**Moore, W. S.**
**1995.** Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.

**Nei, M., and W. -H. Li.**
**1979.** Mathematical model for studying genetic variation

in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76:5269–5273.

**Nei, M., and J. C. Miller.**
**1990.** A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics 125:873–879.

**Nei, M., and F. Tajima.**
**1981.** DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.
**1983.** Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105:207–217.

**Norusis, M. L.**
**1988.** SPSS-X advanced statistics guide, 2nd ed. SPSS Inc., Chicago, IL, 525 p.

**Perrotta, R. G.**
**1993.** Comparacion mediante el empleo de los caracteres meristicos y el crecimiento de caballas originarias de varias regiones geograficas (Cataluña, Islas Canarias y Sudamerica). INIDEP Doc. Cient. 2:7–17.

**Perrotta, R. G., and A. Aubone.**
**1991.** De nuevo sobre la morfometria de la caballa (*Scomber japonicus*). Frente Maritimo 8, sec. A:37–41.

**Roff, D. A., and P. Bentzen.**
**1989.** The statistical analysis of mitochondrial DNA polymorphisms: chi-square and the problem of small samples. Mol. Biol. Evol. 6:539–545.

**Rosenblatt, R. H.**
**1963.** Some aspects of speciation in marine shore fishes. Syst. Assoc. Publ. 5:171–180.

**Rosenblatt, R. H., and R. S. Waples.**
**1986.** A genetic comparison of shore fishes from the eastern and central Pacific Ocean: dispersal or vicariance? Copeia 1986:275–284.

**Sato, Y.**
**1990.** Common mackerel (*Scomber japonicus* Houttuyn) of the Pacific: its ecology and fishing activities. Mar. Behav. Physiol. 17:15–65.

**Scoles, D. R.**
**1994.** Global phylogeography of yellowfin tuna, *Thunnus albacares*, and mackerels of the genus *Scomber*. Ph.D. diss., School of Marine Science, College of William and Mary, VA, 104 p.

**Scoles, D. R., and J. E. Graves.**
**1993.** Genetic analysis of the population structure of yellowfin tuna, *Thunnus albacares*, from the Pacific Ocean. Fish. Bull. 91:690–698.

**Sette, O. E.**
**1950.** Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part II. Migrations and habits. U.S. Fish Wildl. Serv. Fish. Bull. 51:251–358.

**Shaklee, J. B.**
**1984.** Genetic variation and population structure in the damselfish, *Stegastes fasciolatus*, throughout the Hawaiian Archipelago. Copeia 1984:629–640.

**Shulman, M. J., and E. Bermingham.**
**1995.** Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49:897–910.

**Simard, P., M. Castonguay, D. D'Amours, and P. Magnam.**
**1992.** Growth comparison between juvenile Atlantic mackerel (*Scomber scombrus*) from the two spawning groups of the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 49: 2242–2248.

**Sinclair, M.**
**1988.** Marine populations: an essay on population regulation and speciation. Univ. Washington Press. Seattle, WA, 252 p.

**Starks, E. C.**
**1921.** An inconsistency in taxonomy. Science (Wash., D.C.) 54:222–223.

**Stepien, C. A., and R. H. Rosenblatt**
**1996.** Genetic divergence in antitropical pelagic marine fishes (*Trachurus*, *Merluccius*, and *Scomber*) between North and South America. Copeia 1996:586–598.

**Swofford, D. L., and G. J. Olsen.**
**1990.** Phylogeny reconstruction. *In* D. M. Hills and C. Moritz (eds.), Molecular systematics, p 411–501. Sinauer Associates, Inc., Sunderland, MA.

**Thresher, R. E. and E. B. Brothers.**
**1985.** Reproductive ecology and biogeography of Indo-West Pacific angelfishes (Pisces: Pomacanthidae). Evolution 39:878–887.

**Vawter, A. T., R. H. Rosenblatt, and G. C. Gorman.**
**1980.** Genetic divergence among fishes of the eastern Pacific and Caribbean: support for the molecular clock. Evolution 34:705–711.

**Walsh, M., and A. D. F. Johnstone.**
**1992.** Spawning behaviour and diel periodicity of egg production in captive Atlantic mackerel, *Scomber scombrus* L. J. Fish Biol. 40:939–950.

**Ware, D. M. and T. C. Lambert.**
**1985.** Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 42:577–592.

**Watanabe, T.**
**1970.** Morphology and ecology of early life in Japanese common mackerel, *Scomber japonicus*, Houttuyn, with special reference to fluctuation of population. Bull. Tokai Region. Fish. Res. Lab. 62, 283 p.

**Watson, J. J., I. G. Priede, P. R. Witthames, and A. Owori-Wadunde.**
**1992.** Batch fecundity of Atlantic mackerel, *Scomber scombrus* L. J. Fish Biol. 40:591–598.

**Weiss, R.**
**1980.** Investigations on the population differentiation of Pacific *Scomber japonicus*, Houttuyn off NW Africa. Fischerei-Forschung 18:11–14.

**Winans, G. A.**
**1980.** Geographic variation in the milkfish *Chanos chanos*. I. Biochemical evidence. Evolution 34:558–74.

**Zenkin, V. S., and S. N. Lobov.**
**1989.** Genetic-biochemical and phenetic studies of the mackerel (*Scomber japonicus*) population in the Southeast Atlantic (divisions 1.3–1.5). Collect. Sci. Pap. ICSEAF/Recl. Doc. Sci. CIPASE/Colecc. Doc. Cient. Cipaso. Vol. 16(2):197–211.

# Population structure and stock identification of British Columbia coho salmon, *Oncorhynchus kisutch*, based on microsatellite DNA variation

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**Abstract.**—We used genetic variation at three microsatellite DNA loci to describe population structure in 34 coho salmon populations from British Columbia and to perform stock composition analysis on simulated mixed-stock fishery samples. Each microsatellite locus was highly polymorphic, with 31 alleles at *Ots*101, 20 alleles at *Ots*3, and 38 alleles at *Ots*103. Average observed heterozygosities were 86.3%, 73.3% and 74.9%, respectively. Analysis of genetic distances revealed three relatively homogeneous, geographically based groups of coho salmon populations in the following regions: the upper Skeena and Nass River watersheds, the lower Fraser River drainage, and the upper Fraser River-Thompson River watersheds. Coastal populations from the mainland of British Columbia, Vancouver Island, and the Queen Charlotte Islands formed a more heterogeneous regional stock grouping. Significantly different allele frequencies were observed among populations within regions, and allele frequencies were generally temporally stable in multiyear samples. Phylogenetic lineages within British Columbia coho salmon likely reflect geographic patterns of recolonization from at least three separate glacial refugia after the last ice age. Local spawning populations within regions may form metapopulations, but current levels of gene flow among subpopulations are apparently insufficient to prevent differentiation at neutral genetic loci. Maximum-likelihood estimates of stock composition were accurate and precise, indicating great potential for management of coho salmon at the level of metapopulations or "evolutionarily significant units" in domestic and international mixed-stock fisheries. Individual fish were identified to stock by using a discriminant analysis with a high degree of accuracy in a few regions, but more generally with approximately 50% success.

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Of the five commercially important Pacific salmon (*Oncorhynchus*) species exploited in the commercial, recreational and aboriginal fisheries of British Columbia, coho salmon is the one that has shown the largest decline in abundance in both historical (the past 100 years) and recent (the past 40 years) times (Northcote and Atagi, 1997). Coho salmon abundance has also declined dramatically in Washington State, Oregon, and California, to the extent that a number of coho salmon populations, including all of those from the upper Columbia River system, are considered extinct and others are considered endangered (Nehlsen et al., 1991; Weitkamp et al., 1995). The list of factors that may have contributed to declining coho salmon abundance is long, and includes overfishing, loss of freshwater habitat, misguided enhancement efforts, limited marine carrying capacity, and climate changes resulting in altered oceanic conditions (Fraser et al., 1982; Beamish and Bouillon, 1993; Walters, 1993; Reisenbichler, 1997).

Because coho salmon tend to spawn in small streams and rivers that are especially vulnerable to degradation through human activities, freshwater habitat degradation has been considered a major factor in declining coho abundance (Fraser et al., 1982). Similarly, coho salmon commonly are believed to be overexploited because of the mixed-stock nature of most coho salmon fisheries and the frequent bycatch of coho salmon in fisheries directed toward other species (Fraser et al., 1982). Nevertheless, the consistent long-term decline in coho abundance, which has occurred over a broad geographic range in habitats both degraded and pristine and under a range of exploitation levels (Weitkamp et al., 1995; Northcote and Atagi, 1997), indicates that changes occurring in the marine environment are also having a major negative influence on coho salmon populations. This seems especially plausible because in some regions the recent decline in coho abundance coincides with a stable number or increase in abundance of other heavily freshwater-dependent salmonids, such as sockeye salmon and stream-type chinook salmon (Northcote and Atagi, 1997). Thus, coho salmon populations may be less amenable to rebuilding through local or regional habitat improvement and harvest management than populations of other species.

The increasing conservation concerns for coho salmon have led to substantial efforts to quantify

biodiversity in the species (Bartley et al., 1992; Forbes et al., 1993; Weitkamp et al., 1995; Miller and Withler, 1997), to define the appropriate geographic scale of management to conserve the observed biodiversity (Weitkamp et al., 1995; McPhail, 1997; Small et al., 1998), and to develop stock composition methods that would enable the geographically based management required for conservation efforts (Bartley et al., 1992; Beacham et al., 1996; Miller et al., 1996; Van Doornik et al., 1996; Small et al., 1998). Coho salmon populations of California, Oregon, Washington, and southern British Columbia have been categorized into evolutionarily significant units (ESUs), that is to say, reproductively isolated populations or groups of populations that represent the important phylogenetic components of genetic variability in the species (Waples, 1991; Weitkamp et al., 1995). The use of ESUs to delineate biodiversity in Pacific salmon is concordant with an emerging model of metapopulation structure that may supersede the stock concept that has long been applied to these species. Local spawning groups of salmon are no longer viewed as independent and persistent locally adapted "stocks" (Ricker, 1972), but rather as interrelated components, or subpopulations, of a geographically based cluster of such components (the metapopulation) that share a relatively recent evolutionary history and among which gene flow still occurs (McPhail, 1997). Although adaptive differences may arise among the subpopulations of a metapopulation as the result of an appropriate balance between migration and natural selection within subpopulations, they are unlikely to persist over evolutionary time scales as subpopulations go extinct or are swamped by gene flow from adjacent subpopulations. Given this model of salmonid population structure, it is individual metapopulations (ESUs), rather than individual stocks, that managers must conserve to provide the reservoirs of genetic diversity for future evolution within the species.

In coho salmon, and other Pacific salmonids, there is accumulating evidence that the dominant influence on population structure has been the pattern of dispersal from isolated glacial refugia after the last ice age (Gharrett et al., 1987; Wood et al., 1994; Bickham et al., 1995, Miller and Withler 1997; Small et al., 1998). The distinct phylogenetic lineages that can be traced with molecular markers reveal patterns of recolonization of freshwater habitat from refugia located in the Columbia River drainage (Cascadia), the Bering Sea-Yukon River (Beringia), and coastal refugia that likely existed in British Columbia, as well as in more southern waters. For coho, chinook and sockeye salmon, many of these genetically distinct intraspecific lineages converge in British Columbia, providing us with the opportunity and challenge of conserving biodiversity *in situ*.

In this study, we examine variation among and within major river systems and coastal regions in British Columbia at three coho salmon microsatellite loci: *Ots*101, *Ots*3, and *Ots*103. Microsatellite DNA loci consist of highly variable single locus markers containing tandemly repeated arrays of noncoding 1–6 basepair core sequences (Tautz, 1989). For each locus, variation in the number of core sequences creates alleles differing in size by multiples of core-unit length. The rapid rate of mutation and high heterozygosity that characterize microsatellite loci have made them the molecular tool of choice in studies of population structure in vertebrates, including salmonid fish (Angers et al., 1995; McConnell et al., 1995; Scribner et al., 1996; Beacham et al., 1998; Small et al., 1998). In our study, we demonstrate the dual utility of microsatellite variation in defining the regionally based phylogenetic lineages of coho salmon and in accurately detecting their presence in mixed-stock fisheries for management purposes.

## Methods

### DNA samples and PCR

Adult coho salmon tissue samples were collected from 34 coho salmon populations in British Columbia (Fig. 1). Several populations were sampled in multiple years. See Miller et al., (1996) for descriptions of purified genomic DNA extractions from tissues collected up to 1993. Genomic DNA samples from 1994 and 1995 were extracted by using a chelex resin protocol (Small et al., 1998). Microsatellite alleles at three loci were polymerase chain reaction (PCR)-amplified (Sakai et al., 1985) from DNA samples by using primers for the tetranucleotide microsatellites *Ots*101 and *Ots*103 (Small et al., 1998) and the dinucleotide microsatellite, *Ots*3 (Banks[1]). Primer sequences, the PCR conditions, and size-fractionation of the PCR products are described in Small et al. (1998). PCR products from standard test fish were included on each gel to estimate the precision of allele sizing among gels.

### DNA band analysis

Gels were scanned with a Kodak charge-coupled device camera and the images were analyzed as outlined in Small et al. (1998) with BioImage Whole

[1] Banks, M. 1995. Bodega Marine Laboratory, POB 247, Bodega Bay, CA 94923. Personal commun.

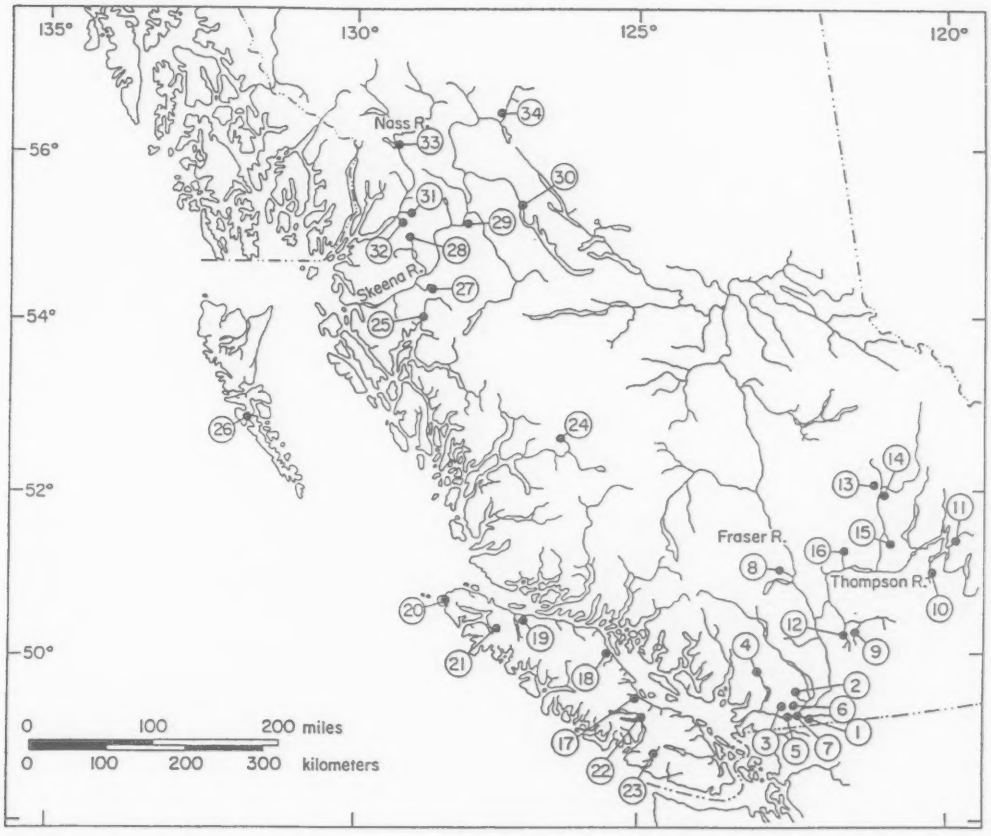


**Figure 1**

Map of British Columbia showing locations of coho salmon samples. Population numbers are placed near the waterway (region or river system name in bold type precedes population names) : **lower Fraser River** 1) Chilliwack River; 2) Chehalis River; 3) Stave River; 4) Upper Pitt River; 5) Nicomen Slough; 6) Norrish Creek; 7) Inch Creek; **upper Fraser River** 8) Bridge River; **Thompson River** 9) Coldwater River; 10) Salmon River; 11) Eagle River; 12) Spius Creek; 13) Lemieux Creek; 14) Dunn Creek; 15) Louis River; 16) Deadman River; **East Coast Vancouver Island** 17) Big Qualicum River; 18) Quinsam River; **North Coast Vancouver Island** 19) Cluxewe River; 20) Stephens Creek; 21) Waukwaas Creek; **West Coast Vancouver Island** 22) Robertson Creek; 23) Nitinat River; **Central Coast** 24) Atnarko River; 25) Kitimat River; **Queen Charlotte Island** 26) Pallant Creek; **Skeena River** 27) Clearwater River; 28) Cedar River; 29) Toboggan Creek; 30) Babine River; 34) Sustut River; **Nass River** 31) Tseax River; 32) Zolzap River; 33) Meziadin River.

Band software (Millipore Corp. Imaging systems, Ann Arbor, MI). The software estimated allele sizes to the nearest basepair with a molecular weight-size-grid created with a 20-bp DNA ladder. Alleles were identified by using a binning procedure (Gill et al., 1990; Galbraith et al., 1991). Allelic bin intervals were created from peaks in allele frequency histograms and precision estimates from standard test fish (Small et al., 1998). Bin intervals for *Ots*101 and *Ots*3 alleles are presented in Table 1 and bin intervals for *Ots*103 alleles are presented in Table 2. The mean bin width was four or more standard deviations from allele sizes of test fish. Allele size was generally four bp for *Ots*101, two bp for *Ots*3, and four bp for *Ots*103. When alleles became infrequent at the extremes of the size ranges and the precision of sizing alleles decreased, alleles were grouped into larger bins (Small et al., 1998). Thirty-one alleles were defined for *Ots*101, 20 alleles were defined for *Ots*3, and 37 alleles (as well as a null allele) were defined for *Ots*103.

## Data analysis

Linkage tests confirmed that the three loci are unlinked and pedigree analysis confirmed Mendelian

**Table 1**

Allele frequencies, observed heterozygosity (Hobs) and expected heterozygosity (Hexp) at *Ots*101 and *Ots*3 loci for coho salmon populations region with region names abbreviated as follows: Central Coast (C Coast), North Coast Vancouver Island (NCVI), West Coast Vancouver Island Populations out of Hardy-Weinberg equilibrium are indicated by * next to the observed heterozygosity. The allele number (in basepairs) is the al. (1998), Table 3. Sample sizes for *Ots*101 are given underneath the population names and for *Ots*3 are indicated by *N*.

| Alleles | **Pallant** 93 | Atnarko 87 | Kitimat 148 | **C Coast** 235 | Cluxewe 38 | Stephens 65 | Waukwaas 30 | **NCVI** 133 | Nitinat 39 | Robertson 84 | **WCVI** 123 | Quinsam 160 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ots*101 | | | | | | | | | | | | |
| 96 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0.065 | **0.045** | 0 |
| 100 | **0.016** | 0.034 | 0.014 | **0.021** | 0.053 | 0.031 | 0.017 | **0.034** | 0 | 0.030 | **0.021** | 0.003 |
| 104 | **0.005** | 0.011 | 0.051 | **0.036** | 0.053 | 0.077 | 0.067 | **0.068** | 0 | 0 | **0** | 0.003 |
| 108 | **0.016** | 0.011 | 0.014 | **0.013** | 0.039 | 0.031 | 0.083 | **0.045** | 0 | 0 | **0** | 0.019 |
| 112 | **0.054** | 0.011 | 0.037 | **0.028** | 0.013 | 0.008 | 0.017 | **0.011** | 0.103 | 0.036 | **0.057** | 0.072 |
| 116 | **0.022** | 0.029 | 0.064 | **0.051** | 0 | 0.023 | 0 | **0.011** | 0 | 0.071 | **0.049** | 0.051 |
| 120 | **0.065** | 0.138 | 0.081 | **0.098** | 0.092 | 0.023 | 0.017 | **0.041** | 0.051 | 0 | **0.016** | 0.081 |
| 124 | **0.141** | 0.092 | 0.088 | **0.088** | 0.171 | 0.131 | 0.101 | **0.135** | 0 | 0.042 | **0.028** | 0.084 |
| 127 | **0.091** | 0.075 | 0.057 | **0.064** | 0.066 | 0.008 | 0.051 | **0.034** | 0 | 0.054 | **0.037** | 0.097 |
| 131 | **0.124** | 0.098 | 0.095 | **0.094** | 0.145 | 0.108 | 0.101 | **0.117** | 0.141 | 0.024 | **0.061** | 0.151 |
| 135 | **0.027** | 0.006 | 0.031 | **0.021** | 0.013 | 0.038 | 0.017 | **0.026** | 0.091 | 0.071 | **0.077** | 0.019 |
| 139 | **0.081** | 0.011 | 0.031 | **0.024** | 0.013 | 0.054 | 0.067 | **0.045** | 0.051 | 0.054 | **0.053** | 0.041 |
| 143 | **0.032** | 0.011 | 0.024 | **0.019** | 0.039 | 0.069 | 0.033 | **0.053** | 0.013 | 0.083 | **0.061** | 0.063 |
| 147 | **0.011** | 0.017 | 0.041 | **0.032** | 0.079 | 0.123 | 0 | **0.083** | 0.205 | 0.149 | **0.167** | 0.022 |
| 151 | **0.022** | 0.011 | 0.010 | **0.011** | 0 | 0.054 | 0.017 | **0.030** | 0.051 | 0.054 | **0.053** | 0.009 |
| 154 | **0.011** | 0.006 | 0.027 | **0.019** | 0.039 | 0.015 | 0.017 | **0.023** | 0.013 | 0 | **0.004** | 0.003 |
| 158 | **0.011** | 0.029 | 0.047 | **0.041** | 0.013 | 0 | 0.017 | **0.008** | 0.077 | 0.012 | **0.033** | 0.019 |
| 161 | **0.027** | 0.034 | 0.027 | **0.031** | 0 | 0.008 | 0.051 | **0.015** | 0.064 | 0.125 | **0.106** | 0.041 |
| 165 | **0** | 0.069 | 0.021 | **0.038** | 0 | 0.023 | 0.051 | **0.023** | 0.026 | 0.048 | **0.041** | 0.059 |
| 169 | **0** | 0.046 | 0.047 | **0.047** | 0.053 | 0 | 0 | **0.015** | 0.038 | 0.048 | **0.045** | 0.069 |
| 173 | **0.048** | 0.040 | 0.057 | **0.051** | 0.026 | 0.008 | 0.101 | **0.034** | 0.013 | 0 | **0.004** | 0.031 |
| 177 | **0.043** | 0.057 | 0.017 | **0.031** | 0.013 | 0.046 | 0.117 | **0.053** | 0.038 | 0.012 | **0.021** | 0.022 |
| 181 | **0.038** | 0.052 | 0.041 | **0.045** | 0.039 | 0.054 | 0 | **0.038** | 0 | 0.006 | **0.004** | 0.009 |
| 185 | **0** | 0.034 | 0.037 | **0.036** | 0.026 | 0.038 | 0 | **0.026** | 0 | 0.012 | **0.008** | 0.019 |
| 189 | **0** | 0.040 | 0.021 | **0.028** | 0.013 | 0 | 0 | **0.004** | 0 | 0.006 | **0.004** | 0 |
| 193 | **0.005** | 0.006 | 0.021 | **0.015** | 0 | 0.015 | 0.017 | **0.011** | 0 | 0 | **0** | 0 |
| 197 | **0.027** | 0 | 0 | **0** | 0 | 0 | 0.017 | **0.004** | 0 | 0 | **0** | 0 |
| 201 | **0.016** | 0.011 | 0 | **0.004** | 0 | 0 | 0.017 | **0.004** | 0 | 0 | **0** | 0.009 |
| 205 | **0.032** | 0.011 | 0.003 | **0.006** | 0 | 0 | 0.017 | **0.004** | 0.013 | 0 | **0.004** | 0.003 |
| 210 | **0.016** | 0.006 | 0.003 | **0.004** | 0 | 0.015 | 0 | **0.008** | 0 | 0 | **0** | 0 |
| 215 | **0.032** | 0.011 | 0 | **0.004** | 0 | 0 | 0 | **0** | 0.013 | 0 | **0.004** | 0.003 |
| Hobs | **0.861** | *0.862 | 0.909 | **0.885** | 0.921 | 0.877 | 0.967 | **0.909** | 0.718 | 0.869 | **0.821** | 0.900 |
| Hexp | **0.935** | 0.943 | 0.946 | **0.945** | 0.921 | 0.938 | 0.933 | **0.939** | 0.897 | 0.940 | **0.927** | 0.931 |
| *Ots*3 | | | | | | | | | | | | |
| *N* | 93 | 86 | 145 | 231 | 38 | 68 | 32 | 138 | 40 | 84 | 124 | 160 |
| 66 | **0.129** | 0.052 | 0.045 | **0.048** | 0 | 0.029 | 0.109 | **0.040** | 0.013 | 0.012 | **0.012** | 0 |
| 68 | **0.005** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 70 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 72 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 74 | **0.011** | 0 | 0.007 | **0.004** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 76 | **0** | 0 | 0.003 | **0.002** | 0.013 | 0 | 0 | **0.004** | 0 | 0 | **0** | 0 |
| 78 | **0** | 0.035 | 0.003 | **0.015** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 82 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 84 | **0** | 0 | 0.014 | **0.009** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0.025 |
| 86 | **0.027** | 0.285 | 0.124 | **0.184** | 0.079 | 0.051 | 0.063 | **0.062** | 0.112 | 0.024 | **0.052** | 0.125 |
| 88 | **0.258** | 0.215 | 0.328 | **0.286** | 0.316 | 0.191 | 0.234 | **0.236** | 0.275 | 0.179 | **0.211** | 0.281 |
| 90 | **0.071** | 0.017 | 0.003 | **0.009** | 0.013 | 0.059 | 0.047 | **0.043** | 0 | 0.221 | **0.149** | 0.034 |
| 92 | **0.011** | 0.012 | 0.066 | **0.045** | 0.092 | 0.141 | 0.078 | **0.112** | 0.151 | 0.101 | **0.117** | 0.019 |
| 94 | **0.323** | 0.251 | 0.238 | **0.242** | 0.368 | 0.419 | 0.234 | **0.362** | 0.138 | 0.292 | **0.242** | 0.338 |
| 96 | **0** | 0.017 | 0.024 | **0.022** | 0.039 | 0.007 | 0.047 | **0.025** | 0 | 0.006 | **0.004** | 0.003 |
| 98 | **0.145** | 0.116 | 0.124 | **0.121** | 0.066 | 0.051 | 0.094 | **0.065** | 0.313 | 0.161 | **0.211** | 0.166 |
| 100 | **0.016** | 0 | 0.003 | **0.002** | 0 | 0.029 | 0.016 | **0.018** | 0 | 0.006 | **0.004** | 0.006 |
| 102 | **0.005** | 0 | 0.017 | **0.011** | 0.005 | 0.078 | 0.013 | **0.033** | 0 | 0 | **0** | 0.003 |
| 104 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| 106 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| Hobs | **0.699** | 0.698 | *0.752 | **0.732** | 0.816 | 0.765 | 0.751 | **0.775** | 0.751 | 0.786 | **0.774** | 0.712 |
| Hexp | **0.785** | 0.790 | 0.800 | **0.809** | 0.763 | 0.765 | 0.875 | **0.789** | 0.775 | 0.798 | **0.814** | 0.762 |

from British Columbia. The weighted mean of allele frequencies for regions (in bold type) follow allele frequencies for individual populations in the (WCVI), East Coast Vancouver Island (ECVI), Skeena River (Skeena), Nass River (Nass), Thompson River (TR), and lower Fraser River (L Fr). lower limit of the allele bin. Allele frequencies for individual populations in the lower Fraser River and the Thompson River are given in Small et

| Big Qualicum 200 | **ECVI** 260 | Toboggan 164 | Cedar 47 | Babine 139 | Clear-water 48 | Sustut 36 | **Skeena** 434 | Tseax 40 | Zolzap 47 | Mexiadin 64 | **Nass** 151 | **TR** 1015 | **L Fr** 1043 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0** |
| 0.011 | **0.007** | 0 | 0 | 0 | 0 | 0 | **0** | 0.013 | 0 | 0 | **0.004** | **0** | **0.018** |
| 0.023 | **0.014** | 0.018 | 0 | 0.029 | 0.010 | 0.028 | **0.020** | 0.013 | 0.011 | 0.063 | **0.022** | **0** | **0.009** |
| 0.025 | **0.022** | 0.015 | 0.053 | 0.068 | 0.063 | 0.056 | **0.045** | 0.025 | 0.096 | 0.078 | **0.059** | **0.001** | **0.037** |
| 0 | **0.032** | 0.003 | 0.021 | 0.004 | 0.021 | 0.042 | **0.010** | 0.025 | 0.021 | 0.031 | **0.026** | **0.001** | **0.011** |
| 0.011 | **0.028** | 0.165 | 0.149 | 0.032 | 0.042 | 0.125 | **0.104** | 0.162 | 0.138 | 0.078 | **0.114** | **0.077** | **0.034** |
| 0.085 | **0.084** | 0.186 | 0.138 | 0.273 | 0.302 | 0.181 | **0.220** | 0.151 | 0.074 | 0.172 | **0.129** | **0.015** | **0.122** |
| 0.081 | **0.082** | 0.049 | 0.138 | 0.137 | 0.031 | 0.056 | **0.084** | 0.101 | 0.096 | 0.078 | **0.088** | **0.001** | **0.082** |
| 0.081 | **0.088** | 0.003 | 0.043 | 0.011 | 0.073 | 0 | **0.017** | 0.051 | 0.011 | 0.078 | **0.037** | **0.007** | **0.112** |
| 0.131 | **0.139** | 0.076 | 0.074 | 0.007 | 0.115 | 0.014 | **0.052** | 0.025 | 0.064 | 0.023 | **0.037** | **0.071** | **0.119** |
| 0.055 | **0.039** | 0.031 | 0.085 | 0.094 | 0.011 | 0.028 | **0.054** | 0.151 | 0.117 | 0.016 | **0.088** | **0.011** | **0.121** |
| 0.063 | **0.053** | 0.006 | 0.043 | 0.011 | 0.031 | 0 | **0.014** | 0.025 | 0.021 | 0.023 | **0.022** | **0.014** | **0.073** |
| 0.025 | **0.042** | 0 | 0.053 | 0 | 0.031 | 0 | **0.009** | 0.013 | 0.021 | 0.016 | **0.011** | **0** | **0.042** |
| 0.018 | **0.019** | 0.003 | 0.043 | 0 | 0.021 | 0.028 | **0.010** | 0.075 | 0.043 | 0.031 | **0.051** | **0.002** | **0.048** |
| 0.027 | **0.019** | 0.006 | 0.011 | 0.004 | 0.031 | 0 | **0.008** | 0.013 | 0.032 | 0 | **0.015** | **0.019** | **0.027** |
| 0.035 | **0.021** | 0.006 | 0 | 0 | 0.011 | 0 | **0.003** | 0 | 0.106 | 0 | **0.037** | **0.051** | **0.021** |
| 0.013 | **0.015** | 0 | 0 | 0.004 | 0 | 0.014 | **0.002** | 0 | 0.011 | 0.008 | **0.007** | **0.011** | **0.011** |
| 0.018 | **0.028** | 0 | 0.011 | 0 | 0.042 | 0.028 | **0.008** | 0.013 | 0.021 | 0 | **0.011** | **0.081** | **0.033** |
| 0.058 | **0.058** | 0 | 0.043 | 0 | 0.021 | 0.014 | **0.008** | 0 | 0 | 0.039 | **0.015** | **0.056** | **0.011** |
| 0.045 | **0.054** | 0.031 | 0 | 0.022 | 0.011 | 0 | **0.020** | 0.050 | 0.021 | 0.016 | **0.029** | **0.159** | **0.005** |
| 0.051 | **0.041** | 0.064 | 0.021 | 0.141 | 0.063 | 0.014 | **0.075** | 0 | 0.064 | 0.055 | **0.048** | **0.111** | **0.011** |
| 0.005 | **0.013** | 0.046 | 0.011 | 0.051 | 0.021 | 0.069 | **0.041** | 0 | 0 | 0.086 | **0.040** | **0.086** | **0.005** |
| 0.005 | **0.007** | 0.031 | 0.011 | 0.018 | 0.031 | 0.111 | **0.030** | 0.013 | 0.011 | 0.047 | **0.029** | **0.091** | **0.006** |
| 0.013 | **0.015** | 0.052 | 0.032 | 0.058 | 0.052 | 0.097 | **0.055** | 0.038 | 0 | 0 | **0.011** | **0.076** | **0.009** |
| 0.013 | **0.007** | 0.119 | 0.011 | 0.029 | 0 | 0.056 | **0.060** | 0.038 | 0.011 | 0.055 | **0.040** | **0.026** | **0.013** |
| 0.027 | **0.015** | 0.082 | 0 | 0.011 | 0 | 0.042 | **0.038** | 0.025 | 0.011 | 0.008 | **0.015** | **0.017** | **0.008** |
| 0.035 | **0.019** | 0.009 | 0 | 0 | 0.021 | 0 | **0.006** | 0 | 0 | 0 | **0** | **0.006** | **0.011** |
| 0.011 | **0.011** | 0.003 | 0.011 | 0 | 0 | 0 | **0.002** | 0.013 | 0.011 | 0.016 | **0.015** | **0.007** | **0.003** |
| 0.045 | **0.026** | 0 | 0 | 0.004 | 0.011 | 0 | **0.002** | 0 | 0 | 0 | **0** | **0.001** | **0.001** |
| 0 | **0** | 0.003 | 0 | 0 | 0 | 0 | **0.001** | 0 | 0 | 0 | **0** | **0.001** | **0.001** |
| 0 | **0.001** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0.001** | **0** |
| 0.885 | **0.889** | 0.835 | 0.809 | 0.853 | 0.813 | 0.806 | **0.822** | 0.851 | 0.883 | 0.844 | **0.821** | **0.881** | **0.874** |
| 0.935 | **0.936** | 0.902 | 0.915 | 0.871 | 0.915 | 0.892 | **0.902** | 0.925 | 0.936 | 0.718 | **0.914** | **0.915** | **0.915** |
| 191 | 351 | 161 | 48 | 139 | 47 | 37 | 432 | 39 | 46 | 52 | 137 | 1016 | 1048 |
| 0.065 | **0.036** | 0.012 | 0.021 | 0.108 | 0 | 0.068 | **0.047** | 0.013 | 0.033 | 0.048 | **0.033** | **0** | **0.034** |
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0** |
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.001** |
| 0.003 | **0.001** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0** |
| 0 | **0** | 0.003 | 0 | 0 | 0 | 0 | **0.001** | 0 | 0 | 0 | **0** | **0** | **0** |
| 0.011 | **0.006** | 0.003 | 0 | 0 | 0 | 0 | **0.001** | 0 | 0 | 0 | **0** | **0** | **0** |
| 0 | **0** | 0 | 0 | 0 | 0.011 | 0 | **0.002** | 0 | 0.022 | 0 | **0.007** | **0** | **0** |
| 0.005 | **0.003** | 0 | 0.011 | 0 | 0 | 0 | **0.002** | 0 | 0 | 0 | **0** | **0** | **0.001** |
| 0.016 | **0.021** | 0.006 | 0.031 | 0 | 0 | 0.054 | **0.010** | 0.051 | 0.011 | 0.011 | **0.022** | **0** | **0.004** |
| 0.102 | **0.113** | 0.065 | 0.229 | 0.111 | 0.128 | 0.271 | **0.132** | 0.154 | 0.272 | 0.125 | **0.182** | **0.011** | **0.082** |
| 0.209 | **0.241** | 0.181 | 0.156 | 0.227 | 0.181 | 0.054 | **0.182** | 0.103 | 0.174 | 0.163 | **0.150** | **0.125** | **0.249** |
| 0.045 | **0.041** | 0.102 | 0 | 0.065 | 0.053 | 0.041 | **0.068** | 0.077 | 0.076 | 0.067 | **0.073** | **0.078** | **0.051** |
| 0.071 | **0.047** | 0.009 | 0.021 | 0.007 | 0.011 | 0.108 | **0.019** | 0.167 | 0.011 | 0.115 | **0.095** | **0.235** | **0.114** |
| 0.262 | **0.296** | 0.363 | 0.292 | 0.223 | 0.319 | 0.203 | **0.292** | 0.218 | 0.185 | 0.115 | **0.168** | **0.444** | **0.282** |
| 0.063 | **0.036** | 0.006 | 0.011 | 0.014 | 0.021 | 0.027 | **0.013** | 0.128 | 0.033 | 0.106 | **0.088** | **0.047** | **0.028** |
| 0.136 | **0.151** | 0.127 | 0.198 | 0.108 | 0.149 | 0.054 | **0.125** | 0.038 | 0.152 | 0.154 | **0.120** | **0.026** | **0.106** |
| 0.013 | **0.011** | 0 | 0 | 0 | 0.021 | 0 | **0.002** | 0.051 | 0.011 | 0 | **0.018** | **0.006** | **0.018** |
| 0.003 | **0.003** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0.029** | **0.031** |
| 0 | **0** | 0.051 | 0.041 | 0 | 0 | 0.014 | **0.037** | 0.011 | 0.058 | 0.032 | **0.026** | **0** | **0** |
| 0 | **0** | 0.071 | 0.068 | 0 | 0 | 0.095 | **0.064** | 0 | 0.038 | 0.064 | **0.015** | **0** | **0** |
| *0.757 | **0.735** | 0.714 | 0.792 | 0.763 | 0.723 | *0.703 | **0.728** | 0.744 | 0.696 | 0.788 | **0.729** | **0.657** | **0.772** |
| 0.848 | **0.815** | 0.801 | 0.812 | 0.849 | 0.796 | 0.865 | **0.826** | 0.850 | 0.812 | 0.885 | **0.864** | **0.722** | **0.822** |

**Table 2**

Uncorrected and corrected (bold type) allele frequencies, observed heterozygosity (Hobs) and expected heterozygosity (Hexp) at *Ots*103 locus frequencies of the populations in the region, with region names abbreviated as in Table 1. Populations out of Hardy-Weinberg equilibrium cies for individual populations in the lower Fraser River and the Thompson River are given in Table 3 of Small et al. (1998). Sample sizes

| Alleles | **Pallant** 93 | Atnarko 87 | **Kitimat** 148 | **C Coast** 235 | Cluxewe 38 | Stephens 65 | Waukwaas 30 | **NCVI** 133 | Nitinat 39 | Robertson 84 | **WCVI** 123 | Quinsam 160 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 61 | **0** | 0 | 0.007 | **0.004** | 0 | 0.037 | 0 | **0.018** | 0.105 | 0 | **0.031** | 0 |
| | **0** | **0** | **0.006** | **0.004** | **0** | **0.037** | **0** | **0.018** | **0.080** | **0** | **0.024** | **0** |
| 71 | **0** | 0 | 0 | **0** | 0.013 | 0.022 | 0 | **0.014** | 0 | 0 | **0** | 0 |
| | **0** | **0** | **0** | **0** | **0.013** | **0.022** | **0** | **0.014** | **0** | **0** | **0** | **0** |
| 75 | **0.218** | 0.039 | 0.081 | **0.064** | 0.184 | 0.265 | 0.156 | **0.217** | 0 | 0.018 | **0.012** | 0.147 |
| | **0.223** | **0.041** | **0.077** | **0.064** | **0.162** | **0.257** | **0.152** | **0.205** | **0** | **0.017** | **0.012** | **0.139** |
| 79 | **0.048** | 0.006 | 0.017 | **0.012** | 0 | 0 | 0.016 | **0.004** | 0.053 | 0.185 | **0.138** | 0.038 |
| | **0.048** | **0.006** | **0.016** | **0.012** | **0** | **0** | **0.016** | **0.004** | **0.050** | **0.161** | **0.124** | **0.035** |
| 83 | **0.021** | 0 | 0 | **0** | 0.026 | 0 | 0 | **0.007** | 0 | 0 | **0** | 0 |
| | **0.021** | **0** | **0** | **0** | **0.026** | **0** | **0** | **0.007** | **0** | **0** | **0** | **0** |
| 86 | **0.005** | 0 | 0.003 | **0.002** | 0.013 | 0.088 | 0.047 | **0.058** | 0.026 | 0.012 | **0.016** | 0 |
| | **0.005** | **0** | **0.003** | **0.002** | **0.013** | **0.089** | **0.033** | **0.053** | **0.025** | **0.011** | **0.016** | **0** |
| 90 | **0** | 0.006 | 0.003 | **0.004** | 0 | 0.007 | 0.016 | **0.007** | 0 | 0.030 | **0.020** | 0.034 |
| | **0** | **0.006** | **0.003** | **0.004** | **0** | **0.007** | **0.016** | **0.007** | **0** | **0.023** | **0.016** | **0.033** |
| 94 | **0** | 0.079 | 0.013 | **0.037** | 0.013 | 0 | 0 | **0.004** | 0.053 | 0 | **0.016** | 0.053 |
| | **0** | **0.075** | **0.013** | **0.035** | **0.013** | **0** | **0** | **0.004** | **0.039** | **0** | **0.012** | **0.051** |
| 98 | **0.005** | 0.067 | 0.010 | **0.031** | 0.026 | 0.059 | 0.031 | **0.043** | 0 | 0.024 | **0.016** | 0 |
| | **0.005** | **0.064** | **0.010** | **0.029** | **0.014** | **0.059** | **0.017** | **0.038** | **0** | **0.023** | **0.016** | **0** |
| 102 | **0.117** | 0.028 | 0.037 | **0.033** | 0.105 | 0 | 0 | **0.029** | 0.039 | 0 | **0.012** | 0.016 |
| | **0.117** | **0.028** | **0.033** | **0.031** | **0.096** | **0** | **0** | **0.026** | **0.038** | **0** | **0.012** | **0.016** |
| 106 | **0.165** | 0.017 | 0.047 | **0.035** | 0.026 | 0.051 | 0.016 | **0.036** | 0.276 | 0.042 | **0.110** | 0.003 |
| | **0.163** | **0.017** | **0.043** | **0.033** | **0.026** | **0.051** | **0.016** | **0.036** | **0.224** | **0.040** | **0.095** | **0.003** |
| 110 | **0.069** | 0.073 | 0.044 | **0.053** | 0 | 0.015 | 0.047 | **0.018** | 0.039 | 0.054 | **0.047** | 0.034 |
| | **0.063** | **0.073** | **0.037** | **0.050** | **0** | **0.015** | **0.033** | **0.015** | **0.026** | **0.037** | **0.033** | **0.029** |
| 114 | **0.027** | 0.051 | 0.034 | **0.037** | 0.013 | 0.044 | 0.125 | **0.054** | 0.026 | 0.060 | **0.043** | 0.009 |
| | **0.027** | **0.042** | **0.029** | **0.034** | **0.013** | **0.039** | **0.125** | **0.052** | **0.025** | **0.047** | **0.040** | **0.009** |
| 117 | **0.005** | 0.006 | 0.060 | **0.039** | 0 | 0.015 | 0.156 | **0.043** | 0 | 0 | **0** | 0 |
| | **0.005** | **0.006** | **0.056** | **0.037** | **0** | **0.015** | **0.124** | **0.034** | **0** | **0** | **0** | **0** |
| 121 | **0.011** | 0 | 0.013 | **0.008** | 0.079 | 0.007 | 0.047 | **0.036** | 0.053 | 0.048 | **0.047** | 0 |
| | **0.011** | **0** | **0.013** | **0.008** | **0.073** | **0.007** | **0.033** | **0.030** | **0.039** | **0.046** | **0.044** | **0** |
| 125 | **0.005** | 0.006 | 0.007 | **0.006** | 0.092 | 0.037 | 0.219 | **0.094** | 0.013 | 0.036 | **0.028** | 0.013 |
| | **0.005** | **0.006** | **0.006** | **0.006** | **0.092** | **0.037** | **0.191** | **0.087** | **0.013** | **0.034** | **0.028** | **0.010** |
| 129 | **0.011** | 0 | 0.020 | **0.012** | 0.066 | 0 | 0 | **0.018** | 0 | 0 | **0** | 0.025 |
| | **0.011** | **0** | **0.017** | **0.010** | **0.054** | **0** | **0** | **0.015** | **0** | **0** | **0** | **0.022** |
| 133 | **0.053** | 0.056 | 0.007 | **0.025** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0.022 |
| | **0.053** | **0.052** | **0.006** | **0.023** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.022** |
| 136 | **0.011** | 0.011 | 0.003 | **0.006** | 0 | 0 | 0 | **0** | 0.039 | 0.006 | **0.016** | 0.041 |
| | **0.011** | **0.011** | **0.003** | **0.006** | **0** | **0** | **0** | **0** | **0.038** | **0.006** | **0.016** | **0.038** |
| 140 | **0** | 0 | 0.003 | **0.002** | 0 | 0.022 | 0 | **0.011** | 0.066 | 0.006 | **0.024** | 0.056 |
| | **0** | **0** | **0.003** | **0.002** | **0** | **0.022** | **0** | **0.011** | **0.052** | **0.006** | **0.020** | **0.054** |
| 144 | **0** | 0.017 | 0.010 | **0.012** | 0 | 0.088 | 0.016 | **0.047** | 0.079 | 0 | **0.024** | 0.022 |
| | **0** | **0.017** | **0.007** | **0.010** | **0** | **0.088** | **0.016** | **0.047** | **0.064** | **0** | **0.020** | **0.022** |
| 148 | **0** | 0.034 | 0.010 | **0.019** | 0.039 | 0.066 | 0 | **0.043** | 0 | 0 | **0** | 0.019 |
| | **0** | **0.034** | **0.010** | **0.019** | **0.039** | **0.066** | **0** | **0.043** | **0** | **0** | **0** | **0.019** |
| 152 | **0** | 0.051 | 0.010 | **0.025** | 0.053 | 0 | 0 | **0.014** | 0 | 0.024 | **0.016** | 0.009 |
| | **0** | **0.039** | **0.010** | **0.019** | **0.053** | **0** | **0** | **0.014** | **0** | **0.023** | **0.016** | **0.009** |
| 156 | **0** | 0.062 | 0.040 | **0.047** | 0.053 | 0.029 | 0.016 | **0.033** | 0 | 0 | **0** | 0.025 |
| | **0** | **0.062** | **0.039** | **0.047** | **0.042** | **0.029** | **0.016** | **0.030** | **0** | **0** | **0** | **0.025** |
| 160 | **0.021** | 0.079 | 0.084 | **0.080** | 0 | 0.015 | 0 | **0.007** | 0.013 | 0 | **0.004** | 0.041 |
| | **0.021** | **0.079** | **0.071** | **0.075** | **0** | **0.015** | **0** | **0.007** | **0.013** | **0** | **0.004** | **0.036** |
| 165 | **0.021** | 0.022 | 0.101 | **0.070** | 0.105 | 0.029 | 0.016 | **0.047** | 0 | 0.006 | **0.004** | 0.159 |
| | **0.021** | **0.022** | **0.086** | **0.063** | **0.087** | **0.024** | **0.016** | **0.038** | **0** | **0.006** | **0.004** | **0.143** |
| 170 | **0.037** | 0.017 | 0.104 | **0.070** | 0.013 | 0.051 | 0.031 | **0.036** | 0.026 | 0.018 | **0.020** | 0.056 |
| | **0.037** | **0.017** | **0.096** | **0.067** | **0.013** | **0.047** | **0.031** | **0.033** | **0.025** | **0.017** | **0.020** | **0.056** |
| 175 | **0.106** | 0.028 | 0.084 | **0.062** | 0.013 | 0 | 0 | **0.004** | 0 | 0.036 | **0.024** | 0.013 |
| | **0.105** | **0.023** | **0.071** | **0.054** | **0.013** | **0** | **0** | **0.004** | **0** | **0.029** | **0.020** | **0.013** |
| 180 | **0.027** | 0.067 | 0.027 | **0.041** | 0.013 | 0 | 0 | **0.004** | 0.013 | 0 | **0.004** | 0.022 |
| | **0.027** | **0.060** | **0.026** | **0.038** | **0.013** | **0** | **0** | **0.004** | **0.013** | **0** | **0.004** | **0.022** |
| 185 | **0** | 0.028 | 0.020 | **0.023** | 0 | 0 | 0.016 | **0.004** | 0 | 0.179 | **0.118** | 0.025 |
| | **0** | **0.028** | **0.019** | **0.023** | **0** | **0** | **0.016** | **0.004** | **0** | **0.147** | **0.097** | **0.022** |
| 190 | **0** | 0.039 | 0.027 | **0.031** | 0.013 | 0.015 | 0 | **0.011** | 0.026 | 0.208 | **0.146** | 0.047 |
| | **0** | **0.039** | **0.026** | **0.031** | **0.013** | **0.015** | **0** | **0.011** | **0.025** | **0.185** | **0.132** | **0.045** |
| 200 | **0.005** | 0.028 | 0.027 | **0.027** | 0.026 | 0.015 | 0 | **0.014** | 0.013 | 0.006 | **0.008** | 0.063 |
| | **0.005** | **0.018** | **0.026** | **0.023** | **0.026** | **0.016** | **0** | **0.015** | **0.013** | **0.006** | **0.008** | **0.058** |
| 210 | **0.005** | 0.062 | 0.030 | **0.041** | 0.013 | 0.007 | 0.016 | **0.011** | 0 | 0 | **0** | 0.003 |
| | **0.005** | **0.058** | **0.029** | **0.040** | **0.013** | **0.007** | **0.016** | **0.011** | **0** | **0** | **0** | **0.003** |
| 220 | **0** | 0.011 | 0.010 | **0.010** | 0 | 0.015 | 0.016 | **0.011** | 0 | 0.006 | **0.004** | 0.003 |
| | **0** | **0.011** | **0.010** | **0.010** | **0** | **0.011** | **0.016** | **0.084** | **0** | **0.006** | **0.004** | **0.003** |
| 230 | **0** | 0.011 | 0.007 | **0.008** | 0 | 0 | 0 | **0** | 0.039 | 0 | **0.012** | 0 |
| | **0** | **0.005** | **0.006** | **0.008** | **0** | **0** | **0** | **0** | **0.026** | **0** | **0.008** | **0** |
| 250 | **0** | 0 | 0 | **0** | 0 | 0 | 0 | **0** | 0 | 0 | **0** | 0 |
| | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| 270 | **0.011** | 0 | 0.003 | **0.002** | 0 | 0 | 0 | **0** | 0 | 0.006 | **0.004** | 0.003 |
| | **0.006** | **0** | **0.003** | **0.002** | **0** | **0** | **0** | **0** | **0** | **0.006** | **0.004** | **0.001** |
| **Null** | **0** | 0 | 0.032 | **0.021** | 0 | **0** | 0 | **0** | 0.050 | 0.034 | **0.039** | 0 |
| | **0.006** | **0.069** | **0.092** | **0.079** | **0.104** | **0.035** | **0.136** | **0.154** | **0.176** | **0.124** | **0.154** | **0.062** |
| Hobs | **0.856** | 0.764 | *0.779 | **0.849** | *0.697 | 0.831 | *0.625 | **0.885** | *0.632 | *0.714 | **0.705** | *0.747 |
| Hexp | **0.777** | 0.791 | 0.948 | **0.979** | 0.921 | 0.897 | 0.906 | **0.920** | 0.899 | 0.885 | **0.959** | 0.931 |

for coho salmon populations from British Columbia. The weighted means of allele frequencies for regions are in bold and follow the are indicated by * next to the observed heterozygosity. The allele number (in basepairs) is the lower limit of the allele bin. Allele frequen- are given underneath the population names.

| Big Qualicum 200 | **ECVI** 260 | Toboggan 164 | Cedar 47 | Babine 139 | Clear-water 48 | Sustut 36 | **Skeena** 434 | Tseax 40 | Zolzap 47 | Mexiadin 64 | **Nass** 151 | **TR** 1015 | **L Fr** 1043 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | **0** | 0.079 | 0.020 | 0.072 | 0.024 | 0 | **0.056** | 0.038 | 0.063 | 0.019 | **0.039** | **0.002** | **0.001** |
| **0** | **0** | **0.076** | **0.019** | **0.065** | **0.010** | **0** | **0.052** | **0.038** | **0.055** | **0.015** | **0.037** | **0.001** | **0.001** |
| 0 | **0** | 0 | 0 | 0.004 | 0.024 | 0 | **0.003** | 0 | 0 | 0 | **0** | **0.024** | **0.027** |
| **0** | **0** | **0** | **0** | **0.004** | **0.010** | **0** | **0.002** | **0** | **0** | **0** | **0** | **0.016** | **0.025** |
| 0.136 | **0.136** | 0.201 | 0.265 | 0.040 | 0.146 | 0.167 | **0.144** | 0.075 | 0.115 | 0.144 | **0.114** | **0.016** | **0.086** |
| **0.114** | **0.125** | **0.176** | **0.208** | **0.037** | **0.076** | **0.132** | **0.121** | **0.075** | **0.103** | **0.093** | **0.105** | **0.011** | **0.082** |
| 0.019 | **0.026** | 0.028 | 0.020 | 0.036 | 0.012 | 0 | **0.025** | 0.038 | 0.031 | 0.010 | **0.025** | **0.002** | **0.010** |
| **0.017** | **0.025** | **0.027** | **0.009** | **0.036** | **0.010** | **0** | **0.024** | **0.038** | **0.031** | **0.007** | **0.025** | **0.001** | **0.009** |
| 0 | **0** | 0.003 | 0 | 0.047 | 0.037 | 0 | **0.019** | 0 | 0 | 0 | **0** | **0.001** | **0.023** |
| **0** | **0** | **0.003** | **0** | **0.046** | **0.021** | **0** | **0.018** | **0** | **0** | **0** | **0** | **0.001** | **0.022** |
| 0.045 | **0.024** | 0.097 | 0.029 | 0.086 | 0 | 0.014 | **0.067** | 0.038 | 0.021 | 0.067 | **0.043** | **0.003** | **0.018** |
| **0.038** | **0.021** | **0.091** | **0.028** | **0.080** | **0** | **0.014** | **0.063** | **0.038** | **0.021** | **0.045** | **0.040** | **0.002** | **0.017** |
| 0.019 | **0.025** | 0.022 | 0 | 0.036 | 0.037 | 0.014 | **0.024** | 0.025 | 0.042 | 0.067 | **0.046** | **0.001** | **0.056** |
| **0.015** | **0.022** | **0.021** | **0** | **0.036** | **0.021** | **0.014** | **0.023** | **0.014** | **0.042** | **0.045** | **0.043** | **0.001** | **0.053** |
| 0.043 | **0.046** | 0 | 0 | 0 | 0.024 | 0 | **0.002** | 0 | 0 | 0 | **0** | **0.029** | **0.011** |
| **0.040** | **0.045** | **0** | **0** | **0** | **0.010** | **0** | **0.001** | **0** | **0** | **0** | **0** | **0.020** | **0.010** |
| 0.029 | **0.015** | 0 | 0 | 0.014 | 0 | 0 | **0.005** | 0.038 | 0 | 0 | **0.011** | **0.003** | **0.014** |
| **0.027** | **0.015** | **0** | **0** | **0.014** | **0** | **0** | **0.005** | **0.038** | **0** | **0** | **0.011** | **0.003** | **0.014** |
| 0 | **0.007** | 0.003 | 0.049 | 0.011 | 0.012 | 0 | **0.011** | 0.025 | 0.021 | 0 | **0.014** | **0.002** | **0.038** |
| **0** | **0.007** | **0.003** | **0.038** | **0.011** | **0.010** | **0** | **0.010** | **0.025** | **0.021** | **0** | **0.014** | **0.001** | **0.036** |
| 0.005 | **0.004** | 0.013 | 0.010 | 0.119 | 0.085 | 0.069 | **0.057** | 0.050 | 0.010 | 0.029 | **0.029** | **0.049** | **0.034** |
| **0.005** | **0.004** | **0.012** | **0.009** | **0.111** | **0.042** | **0.056** | **0.049** | **0.050** | **0.010** | **0.022** | **0.029** | **0.034** | **0.033** |
| 0.008 | **0.019** | 0.126 | 0.010 | 0.194 | 0.061 | 0.083 | **0.121** | 0.025 | 0.073 | 0.029 | **0.043** | **0.302** | **0.031** |
| **0.007** | **0.017** | **0.109** | **0.009** | **0.181** | **0.051** | **0.070** | **0.107** | **0.014** | **0.066** | **0.022** | **0.037** | **0.209** | **0.028** |
| 0.019 | **0.014** | 0.006 | 0.069 | 0.004 | 0.110 | 0.056 | **0.026** | 0.013 | 0.021 | 0.038 | **0.025** | **0.123** | **0.036** |
| **0.017** | **0.014** | **0.006** | **0.057** | **0.004** | **0.063** | **0.029** | **0.020** | **0.014** | **0.021** | **0.030** | **0.025** | **0.080** | **0.035** |
| 0.011 | **0.006** | 0.053 | 0.049 | 0 | 0 | 0 | **0.025** | 0.013 | 0.021 | 0.019 | **0.018** | **0.025** | **0.069** |
| **0.010** | **0.006** | **0.047** | **0.038** | **0** | **0** | **0** | **0.022** | **0.013** | **0.021** | **0.015** | **0.018** | **0.018** | **0.065** |
| 0.005 | **0.003** | 0.006 | 0 | 0 | 0.024 | 0 | **0.005** | 0.038 | 0.031 | 0.019 | **0.029** | **0.012** | **0.019** |
| **0.005** | **0.003** | **0.006** | **0** | **0** | **0.010** | **0** | **0.003** | **0.038** | **0.031** | **0.015** | **0.029** | **0.009** | **0.018** |
| 0.021 | **0.015** | 0 | 0 | 0 | 0.037 | 0 | **0.003** | 0.038 | 0.031 | 0 | **0.021** | **0.035** | **0.033** |
| **0.017** | **0.014** | **0** | **0** | **0** | **0.031** | **0** | **0.003** | **0.038** | **0.031** | **0** | **0.021** | **0.027** | **0.033** |
| 0.021 | **0.021** | 0 | 0 | 0.004 | 0 | 0 | **0.001** | 0 | 0 | 0 | **0** | **0.008** | **0.019** |
| **0.015** | **0.018** | **0** | **0** | **0.004** | **0** | **0** | **0.001** | **0** | **0** | **0** | **0** | **0.005** | **0.018** |
| 0.003 | **0.011** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.006** |
| **0.002** | **0.011** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.006** |
| 0.045 | **0.042** | 0 | 0.010 | 0.011 | 0 | 0 | **0.005** | 0 | 0.010 | 0.010 | **0.007** | **0.002** | **0.037** |
| **0.036** | **0.037** | **0** | **0.009** | **0.011** | **0** | **0** | **0.005** | **0** | **0.010** | **0.007** | **0.007** | **0.002** | **0.036** |
| 0.024 | **0.038** | 0.003 | 0.010 | 0.004 | 0.037 | 0.014 | **0.008** | 0.038 | 0.063 | 0.087 | **0.064** | **0.001** | **0.059** |
| **0.022** | **0.036** | **0.003** | **0.009** | **0.004** | **0.031** | **0.014** | **0.008** | **0.038** | **0.066** | **0.060** | **0.062** | **0.001** | **0.056** |
| 0.016 | **0.018** | 0.016 | 0.010 | 0.011 | 0 | 0 | **0.010** | 0 | 0.042 | 0.029 | **0.025** | **0.006** | **0.058** |
| **0.013** | **0.017** | **0.015** | **0.009** | **0.007** | **0** | **0** | **0.009** | **0** | **0.042** | **0.022** | **0.025** | **0.003** | **0.056** |
| 0.008 | **0.013** | 0.006 | 0.020 | 0.004 | 0.049 | 0 | **0.010** | 0.038 | 0.010 | 0.019 | **0.021** | **0.019** | **0.039** |
| **0.007** | **0.013** | **0.006** | **0.019** | **0.004** | **0.021** | **0** | **0.008** | **0.038** | **0.011** | **0.008** | **0.018** | **0.014** | **0.037** |
| 0.051 | **0.031** | 0.022 | 0 | 0.018 | 0.049 | 0.042 | **0.022** | 0.075 | 0.021 | 0.029 | **0.039** | **0.017** | **0.038** |
| **0.041** | **0.027** | **0.021** | **0** | **0.018** | **0.031** | **0.028** | **0.020** | **0.067** | **0.022** | **0.022** | **0.037** | **0.015** | **0.037** |
| 0.106 | **0.067** | 0.035 | 0.059 | 0.061 | 0.085 | 0.153 | **0.059** | 0.038 | 0.083 | 0.058 | **0.061** | **0.004** | **0.036** |
| **0.088** | **0.060** | **0.031** | **0.056** | **0.058** | **0.042** | **0.117** | **0.052** | **0.038** | **0.077** | **0.038** | **0.056** | **0.011** | **0.036** |
| 0.061 | **0.050** | 0.041 | 0.010 | 0.036 | 0.037 | 0 | **0.031** | 0.100 | 0.031 | 0.077 | **0.068** | **0.008** | **0.026** |
| **0.044** | **0.041** | **0.037** | **0.009** | **0.036** | **0.031** | **0** | **0.030** | **0.093** | **0.031** | **0.046** | **0.061** | **0.003** | **0.025** |
| 0.056 | **0.100** | 0.009 | 0.029 | 0.004 | 0 | 0.014 | **0.009** | 0.063 | 0.021 | 0.029 | **0.036** | **0.020** | **0.035** |
| **0.044** | **0.085** | **0.009** | **0.028** | **0.004** | **0** | **0.014** | **0.009** | **0.063** | **0.021** | **0.022** | **0.036** | **0.006** | **0.033** |
| 0.053 | **0.053** | 0.003 | 0.029 | 0.025 | 0.073 | 0.042 | **0.023** | 0.025 | 0.021 | 0.019 | **0.021** | **0.065** | **0.036** |
| **0.045** | **0.051** | **0.003** | **0.028** | **0.025** | **0.042** | **0.041** | **0.021** | **0.025** | **0.021** | **0.008** | **0.018** | **0.045** | **0.035** |
| 0.056 | **0.035** | 0.025 | 0.088 | 0.004 | 0.024 | 0.069 | **0.029** | 0.038 | 0.042 | 0.010 | **0.029** | **0.008** | **0.040** |
| **0.050** | **0.034** | **0.024** | **0.076** | **0.004** | **0.020** | **0.043** | **0.025** | **0.038** | **0.042** | **0.007** | **0.029** | **0.005** | **0.040** |
| 0.043 | **0.032** | 0.031 | 0.029 | 0.018 | 0.012 | 0.056 | **0.026** | 0.038 | 0.021 | 0.048 | **0.036** | **0.007** | **0.019** |
| **0.035** | **0.029** | **0.030** | **0.028** | **0.015** | **0.010** | **0.042** | **0.024** | **0.038** | **0.021** | **0.037** | **0.036** | **0.005** | **0.019** |
| 0.005 | **0.014** | 0.035 | 0.010 | 0.029 | 0 | 0.028 | **0.025** | 0 | 0.010 | 0.058 | **0.025** | **0** | **0.019** |
| **0.005** | **0.013** | **0.034** | **0.009** | **0.029** | **0** | **0.027** | **0.025** | **0** | **0.010** | **0.045** | **0.025** | **0** | **0.018** |
| 0.061 | **0.051** | 0.085 | 0 | 0.014 | 0 | 0 | **0.035** | 0.087 | 0.073 | 0.019 | **0.057** | **0** | **0.005** |
| **0.050** | **0.048** | **0.078** | **0** | **0.014** | **0** | **0** | **0.033** | **0.071** | **0.066** | **0.015** | **0.049** | **0** | **0.005** |
| 0.027 | **0.042** | 0.022 | 0.039 | 0.018 | 0 | 0 | **0.018** | 0 | 0.042 | 0.010 | **0.018** | **0.004** | **0.004** |
| **0.023** | **0.038** | **0.021** | **0.037** | **0.018** | **0** | **0** | **0.018** | **0** | **0.042** | **0.007** | **0.018** | **0.002** | **0.004** |
| 0.003 | **0.003** | 0.025 | 0.010 | 0.05 | 0 | 0.111 | **0.035** | 0.013 | 0.031 | 0.038 | **0.029** | **0.007** | **0.005** |
| **0.002** | **0.003** | **0.024** | **0.009** | **0.048** | **0** | **0.097** | **0.033** | **0.013** | **0.031** | **0.030** | **0.029** | **0.004** | **0.005** |
| 0 | **0.001** | 0.003 | 0.010 | 0.029 | 0 | 0.069 | **0.017** | 0 | 0 | 0.019 | **0.007** | **0** | **0.002** |
| **0** | **0.001** | **0.003** | **0.009** | **0.029** | **0** | **0.068** | **0.017** | **0** | **0** | **0.015** | **0.005** | **0** | **0.002** |
| 0 | **0** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.001** |
| **0** | **0.003** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.001** |
| 0.005 | **0.003** | 0 | 0.118 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0** |
| **0.005** | **0.004** | **0** | **0.064** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| 0.005 | **0.004** | 0 | 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | **0** | **0** | **0.001** |
| **0.005** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.001** |
| 0.060 | **0.033** | 0.030 | 0.007 | 0 | 0.163 | 0.027 | **0.041** | 0 | 0 | 0 | **0** | **0.193** | **0.007** |
| **0.153** | **0.115** | **0.080** | **0.186** | **0.052** | **0.406** | **0.197** | **0.137** | **0.066** | **0.065** | **0.300** | **0.063** | **0.444** | **0.048** |
| *0.750 | **0.764** | 0.774 | *0.588 | *0.737 | *0.390 | *0.431 | **0.721** | 0.837 | 0.802 | *0.721 | **0.836** | 0.254 | 0.875 |
| 0.945 | **0.944** | 0.915 | 0.896 | 0.921 | 0.938 | 0.919 | **0.938** | 0.950 | 0.958 | 0.942 | **0.957** | **0.086** | **0.958** |

inheritance at all three loci, although a null (nonamplifying) allele is present at *Ots*103 (Small et al., 1998). Individual samples that although amplified with both other primer sets yet produced no *Ots*103 alleles after the *Ots*103 amplification was performed three times, were scored as *Ots*103 null homozygotes. Allele frequencies at the *Ots*103 locus were then corrected according to a maximum-likelihood estimate (EM algorithm, Dempster et al., 1977) by using GENEPOP, version 1.2 (Raymond and Rousset, 1995a). Corrected frequencies were used in the genetic distance analysis. For *Ots*101 and *Ots*3, fish with a single allele were scored as homozygotes and fish with two alleles were scored as heterozygotes. Allele frequency data for individual populations in the Fraser River and Thompson River are from Small et al. (1998) and only the weighted means of total allele frequencies for these regions are presented here (Tables 1 and 2). The upper Fraser River population (Bridge River) has been grouped with the Thompson River populations in accordance with its genetic profile.

Hardy-Weinberg equilibrium (HWE) was tested with the probability test of HWE (an exact HW test, Guo and Thompson, 1992) with GENEPOP (version 1.2). *F*-statistics and their standard deviations were calculated according to Weir and Cockerham (1984) using FSTAT (Goudet, 1995). We use the notations $F_{st}$, $F_{is}$ and $F_{it}$ for Weir and Cockerham's $\theta$, $f$, and $F$, respectively. *P*-values for $\alpha = 0.05$ in all data analyses were corrected for simultaneous multiple tests (Lessios, 1992). Pairwise comparisons of populations for differences in allele distributions were conducted at all three loci with Fisher's exact tests (Raymond and Rousset, 1995b). In populations with multiple year classes, annual variability in allele frequencies was tested with 1000 simulations in a Monte Carlo analysis (Roff and Bentzen, 1989) and populations were divided into year classes for a neighbor-joining (NJ) analysis (Saitou and Nei, 1987). A NJ dendrogram illustrating genetic relationships among populations was constructed with PHYLIP 3.5c software (Felsenstein, 1993). The allele frequency matrix was resampled 500 times and Cavalli-Sforza and Edward's (1967) chord distances were estimated for population pairs. NJ dendrograms were constructed for each matrix and a consensus NJ dendrogram was generated with CONSENSE (see Felsenstein, 1993). Individual fish were classified to specific populations with a jackknife discriminant analysis (SAS Institute Inc., 1989).

## Estimation of stock composition

Microsatellite allele frequency data were examined for use in estimating stock composition in a mixed-stock fishery. We pooled low-frequency alleles in adjacent bins (so that each bin contained at least 6% of the alleles) to reduce the number of genotypes for which frequencies were estimated. This pooling resulted in 13 "analysis" bins (91 genotypes) for *Ots*101, 10 bins (65 genotypes) for *Ots*3, and 13 bins (91 genotypes) for *Ots*103 (Table 3). Genotype frequencies at *Ots*101 and *Ots*3 were estimated for each population from the allele frequencies by assuming a Hardy-Weinberg distribution of genotypes. Because genotype frequencies at the *Ots*103 locus were generally not in HWE owing to the presence of the null allele, the observed genotype frequencies were used to characterize each population for *Ots*103. Genotype frequencies at all three loci were used as input in a maximum likelihood mixed-stock fishery analysis (Fournier et al., 1984).

Two types of hypothetical mixed-stock fishery samples were simulated: single-region fishery samples composed of fish from lower Fraser River and the Thompson River coho salmon populations, and multiregion fishery samples composed of fish from populations from several regions. Populations contributing to the multiregion samples were chosen on the basis of known or inferred migration patterns. For the Fraser River fishery simulation, only populations from the lower Fraser and Thompson rivers were present in the baseline and in the mixed-stock fishery samples. In the multiregion fishery simulations, all 34 populations were used in the baseline. In all simulations, fishery samples of 200 fish were generated from specified populations in the

**Table 3**

Method of pooling low-frequency alleles for *Ots*101, *Ots*3, and *Ots*103 to reduce the number of genotypes for baseline populations in mixed-stock analyses.

| | Microsatellite allele bin numbers | | |
|---|---|---|---|
| Analysis bin no. | *Ots*101 | *Ots*3 | *Ots*103 |
| 1 | 1–7 | 1 | 1, 2, 3 |
| 2 | 8, 9 | 3–11 | 4–7 |
| 3 | 10 | 12 | 8–11 |
| 4 | 11 | 13 | 12 |
| 5 | 12 | 14 | 13 |
| 6 | 13, 14 | 15 | 14, 15 |
| 7 | 15, 16, 17 | 16 | 16–20 |
| 8 | 18, 19 | 17 | 21, 22 |
| 9 | 20, 21 | 18 | 23, 24 |
| 10 | 22 | 19 | 25, 26, 27 |
| 11 | 23, 24 | | 28,29 |
| 12 | 25, 26 | | 30–38 |
| 13 | 27–32 | | 39 |

baseline by sampling randomly, with replacement, thus simulating the randomness present in data collection. For each simulated mixed-stock fishery, stock contributions were estimated in 50 independent simulations, and means and standard deviations for the estimated contributions of each population were obtained.

## Results

### Allele frequencies and heterozygosity

Observed heterozygosity was high in all populations at *Ots*101, ranging from 0.72 for Nitinat River to 0.97 for Waukwaas River (Table 1). Heterozygosity was slightly lower at *Ots*3, ranging from 0.70 for Zolzap River to 0.82 for Cluxewe River (Table 1). At *Ots*103, observed heterozygosity varied widely among regions, ranging from 0.25 in the Thompson River (TR) to 0.89 in North Coast Vancouver Island populations (NCVI) (Table 2). For Thompson River populations, the apparently low heterozygosity values were due to high frequencies of the null allele. Of the 22 populations with data for multiple year classes, Atnarko was the only one for which allele frequencies differed significantly among year classes at all loci. Allele frequencies differed significantly among year classes in the Kitimat, Sustut, and Toboggan populations at *Ots*3, in the Kitimat River and Pallant Creek populations at *Ots*101, and in the Quinsam and Toboggan populations at *Ots*103. Year-class variation in the Fraser and Thompson River populations are reported in Small et al. (1998). Multiple samples from individual populations clustered together in NJ analyses (except for three lower Fraser River populations as noted in Small et al., 1998), indicating that allele frequency differences among year classes were less than those among populations. Thus, all fish collected from the same location in different years were pooled and treated as a single population in subsequent analyses.

### Hardy-Weinberg equilibrium

The degree of deviation from HWE varied substantially among the 3 loci (Table 1). After correction for multiple tests ($P<0.0015$), three populations (Atnarko, Deadman and Upper Pitt) deviated from HWE at *Ots*101, and three populations (Kitimat, Big Qualicum, and Sustut) were out of HWE at *Ots*3 (Table 1). In most populations, observed heterozygosity was lower than expected heterozygosity (Table 1), and this lower heterozygosity was reflected in single-locus $F_{is}$ values of 0.0435 (SD 0.009) for *Ots*101 and 0.0617 (SD 0.008) for *Ots*3 (both $P<0.005$). HWE was rejected for *Ots*103 in 25 out of 34 populations (Table 2), reflecting the presence of the null allele. The single locus $F_{is}$ value was 0.2738 (SD 0.008). Fish homozygous for the null allele were scored in most populations and corrected *Ots*103 allele frequencies were generated for all populations (Table 2).

### Population differences

In pairwise tests, all populations had significantly different allele frequencies at one or more loci, with the exception of two geographically proximate populations in the Thompson River (Lemieux River and Dunn Creek), and two sets of populations from the adjacent Skeena and Nass River systems: (Cedar River [Skeena)]and Zolzap River [Nass]; and Sustut River [Skeena] and Meziadin River [Nass]). The single- and multilocus $F_{st}$ values indicated significant differentiation among populations with values of 0.040 (SD 0.006) for *Ots*101, 0.054 (SD 0.009) for *Ots*3, 0.059 (SD 0.009) for *Ots*103 (all $P<0.005$) and a multilocus value of 0.051 (SD 0.006, $P<0.005$).

### Allele frequencies

With the exception of *Ots*101 allele 96, found only in the Robertson Creek population, all alleles were present in more than one population and region. Thus, population- or region-specific alleles were generally nonexistent, but allele frequencies varied among populations and regions. At *Ots*101, lower Fraser River populations had high frequencies of smaller alleles (74% shorter than 143 bp in length) and relatively low frequencies of large alleles, whereas Thompson River populations had low frequencies of small alleles (64% longer than 161 bp). In the Thompson River populations, *Ots*3 allele 66 was absent and allele 94 was more common than in populations from other regions. *Ots*3 alleles 104 and 106 were found only in the Skeena and Nass River populations. The most striking differentiation provided by *Ots*103 was the high frequency (0.44) of the null allele in Thompson River populations. This was three times the next highest frequency (0.15) that occurred in the north and west coast Vancouver Island populations.

### Population structure

The unrooted consensus NJ dendrogram possessed three major branches that provided regional definition among coho salmon populations of British Columbia (Fig. 2). The best defined branch contained the Thompson River (and Bridge River of the upper Fraser drainage) populations, that occurred together

in all 500 trees used to construct the consensus tree. The branch containing all lower Fraser River populations was also well supported. Populations from the northern Skeena and Nass rivers were interspersed on the third branch, but only the upper Skeena and Nass populations formed a well-supported group (Fig. 2). Lower Skeena and Nass populations were more similar to a diverse central cluster of Vancouver Island and mainland coastal coho salmon populations. Within the coastal group, east coast (Big Qualicum and Quinsam), west coast (Robertson and Nitinat), and north coast (Cluxewe, Waukwaas and Stephens) Vancouver Island populations were as well distinguished from each other as they were from the more northern mainland coastal populations or the Queen Charlotte Island population of Pallant Creek.

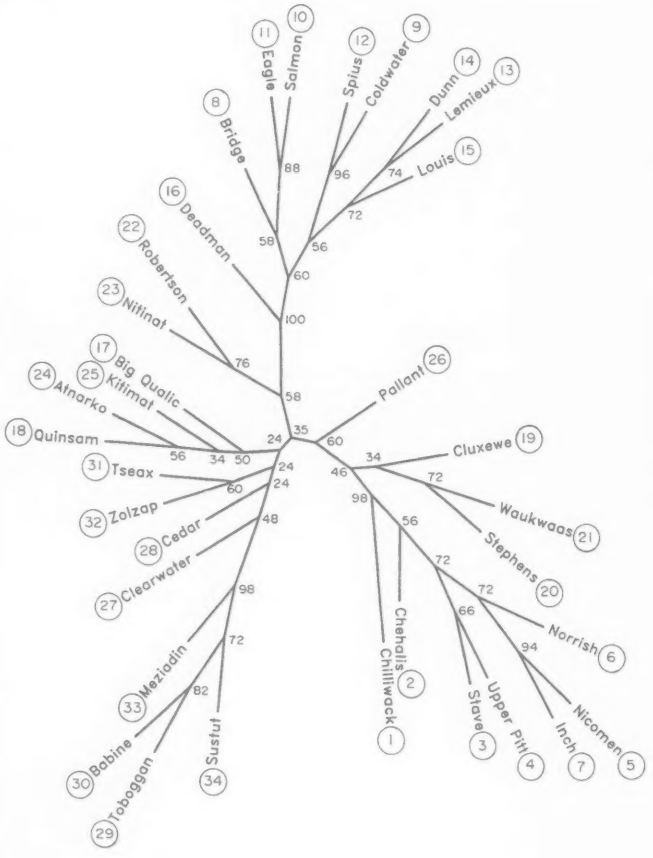


**Figure 2**

Unrooted neighbor-joining tree relating 34 British Columbia coho salmon populations. The tree was constructed from a consensus of Cavalli-Sforza and Edward's chord distances. Bootstrap values at the tree nodes were computed over 500 replications by resampling the allele frequency matrix. Bootstrap values indicate the percentage of trees in which the populations beyond the node occurred together. All names correspond to population names given in Tables 2 and 3 with the exception of Big Qualicum River which is shortened to Big Qualic. The number of the population (from Fig. 1) is next to the name.

## Estimation of stock composition

In the mixed-stock fishery simulated within the Fraser River, estimates of stock composition were accurate and precise, with an average of 2.3% of the mixture incorrectly assigned to each population (Table 4). In general, misassigned portions of the mixture were assigned to closely related populations. For instance, the 30% contribution of Coldwater River fish to the mixture was underestimated as 26.4%, but 3.1% of the mixture was assigned to the genetically similar Spius River population, although no Spius fish were included in the mixture. Fish misassigned to population were almost invariably assigned to the correct region. Less than 1% of fish were misclassified between the Thompson River and Lower Fraser regions (Table 4). Thus, within the Fraser River drainage, populations contributing to a mixed-stock sample can be identified to region and to individual population, or population group within region, with a high degree of accuracy and precision.

Accuracy and precision of stock composition estimates were also high in the simulated mixed-stock samples to which populations from different regions contributed (Table 5). Average error of individual population contributions to the mixed fishery was 1.5% (3% for populations actually contributing to the mixture), and average error of regional contributions was 2.2% (higher for regions actually contributing). In most simulations, the contributions of populations present in the mixture were underestimated because a small proportion of the mixture was allocated to baseline populations not present in the mixture. In general, misassigned fish were allocated to genetically similar populations and thus were

correctly identified to region. Regions with no populations present in the mixture were allocated less than 5% of the fish, except in the mixture composed of Vancouver Island populations (mix 1, Table 5). In that case, 10% of the fish were allocated to the lower Fraser River. The regional misclassification of Vancouver Island coho salmon as fish of lower Fraser origin in mix 1 was due largely to the allocation of 4% of the mixture to Chilliwack River, the lower Fraser population most genetically similar to the northern Vancouver Island populations (Fig. 2). Similarly, Vancouver Island and central coast fish were underestimated in mix 5, and some of them attributed to the lower Fraser rather than to other coastal island, mainland, or lower Skeena and Nass populations as might be expected from the dendrogram (Table 5; Fig. 2). Conversely, when lower Fraser coho salmon formed a high proportion of the mixture (mix 3), they were underestimated and misidentified fish tended to be attributed to Vancouver Island populations (Table 5).

In contrast, the genetic distinctiveness of Thompson River coho salmon resulted in their accurate identification in mixtures in which they were present (mixes 3 and 5) and little misrepresentation in mixtures from which they were absent (mixes 1, 2, and 4). Nass and Skeena populations were well-separated (mix 4), and contributions from lower Skeena (Cedar and Clearwater) populations were identified as well as those from the upper Skeena-Nass populations. Given the lack of separation of Skeena and Nass in the NJ dendrogram (Fig. 2), this result needs to be confirmed by more extensive sampling and further mixture analysis for populations within these two watersheds. Individual classification of Nass and Skeena fish (see below), and the lack of significant allele frequency differences between two Nass-Skeena pairs of populations, indicated that additional genetic markers may be required for accurate differentiation of Nass and Skeena coho salmon. Nevertheless, the results of these preliminary analyses indicate that a mixed-stock sample of coho salmon collected in the field can generally be resolved into its regional components by using microsatellite genetic markers.

### Identifying individuals

Accuracy of the classification of individual fish to region with discriminant analysis varied among regions (Table 6), but individual classification was generally much less accurate than was estimation of stock composition. An average of 48% of fish, ranging from 85% of Thompson to 20% of Nass individuals, was correctly classified to region. Misclassified fish were most commonly attributed to the most genetically similar region according to relationships depicted in the NJ dendrogram. The Nass and Skeena populations were interspersed in the NJ analysis (Fig. 2), and an essentially equal proportion of Nass River fish (20%) were identified as Nass and Skeena fish (Table 6). Skeena River fish were identified more accurately than Nass River fish, with 53% and 11% of the Skeena River fish classified as being of Skeena and Nass origin, respectively. If only the Fraser River populations were included in the baseline, Fraser River coho salmon were assigned to region accurately, with 93% correctly identified to either the lower Fraser River or the Thompson River (data not shown).

**Table 4**

Accuracy and precision of estimated Fraser River coho salmon population contributions in a simulated mixed-stock sample from a 16-population Fraser River baseline. A 200-fish mixture was generated with replacement from the baseline 50 times and the population composition was estimated for each mixture. The mean percentage of the mixture allocated to each population is given, followed by the standard deviation in parentheses. Regional totals are given in the rows marked TR (Thompson River) and L Fr (lower Fraser River).

| | True | Mean | SD |
|---|---|---|---|
| Coldwater | 30 | 26.4 | (4.4) |
| Salmon | 0 | 1.3 | (1.6) |
| Eagle | 20 | 15.7 | (3.4) |
| Spius | 0 | 3.1 | (3.5) |
| Lemieux | 0 | 1.0 | (1.5) |
| Dunn | 0 | 0.6 | (1.3) |
| Louis | 0 | 1.0 | (1.7) |
| Deadman | 0 | 0.5 | (0.9) |
| Bridge | 0 | 0.7 | (1.3) |
| TR | 50 | 50.2 | (1.4) |
| Chilliwack | 20 | 16.8 | (4.0) |
| Chehalis | 0 | 1.4 | (1.8) |
| Stave | 15 | 12.6 | (3.3) |
| Upper Pitt | 0 | 1.8 | (2.1) |
| Nicomen | 15 | 12.2 | (3.4) |
| Norrish | 0 | 2.9 | (2.5) |
| Inch | 0 | 2.0 | (2.4) |
| L Fr | 50 | 49.8 | (1.4) |

## Discussion

Microsatellite DNA analysis shows great promise for the elucidation of population structure in coho salmon. Very strong regional structure was apparent in the British Columbia coho salmon populations

**Table 5**

Estimated stock composition of five 200-fish mixtures of coho salmon from several regions in British Columbia using a 34-stock baseline. Each mixture was generated 50 times, and stock composition of the mixture was estimated by randomly resampling each baseline population, with replacement, to derive a new estimation of the fish mixture composition. The mean and standard deviations of 50 estimations are reported for each population. Individual population estimates are followed by the sum of contributions from a region (bold type).

| | Mix 1 | | | Mix 2 | | | Mix 3 | | | Mix 4 | | | Mix 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | True | Mean | SD | True | Mean | SD | True | Mean | SD | True | Mean | SD | True | Mean | SD |
| **Pallant** | **0** | **0.02** | **0.02** | **0** | **0.01** | **0.01** | **0** | **0.00** | **0.01** | **0** | **0.00** | **0.01** | **0** | **0.01** | **0.01** |
| Atnarko | 0 | 0.01 | 0.01 | 0.13 | 0.11 | 0.02 | 0 | 0.00 | 0.01 | 0 | 0.02 | 0.02 | 0 | 0.02 | 0.02 |
| Kitimat | 0 | 0.01 | 0.02 | 0.13 | 0.10 | 0.02 | 0 | 0.01 | 0.01 | 0 | 0.02 | 0.02 | 0.20 | 0.16 | 0.04 |
| **C Coast** | **0** | **0.02** | **0.02** | **0.26** | **0.21** | **0.03** | **0** | **0.01** | **0.01** | **0** | **0.04** | **0.03** | **0.20** | **0.18** | **0.04** |
| Cluxewe | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.01 | 0.01 |
| Stephens | 0.13 | 0.10 | 0.02 | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.02 |
| Waukwaas | 0.17 | 0.12 | 0.03 | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.01 |
| **NCVI** | **0.30** | **0.23** | **0.03** | **0** | **0.01** | **0.01** | **0** | **0.02** | **0.02** | **0** | **0.01** | **0.01** | **0** | **0.02** | **0.02** |
| Nitinat | 0.13 | 0.10 | 0.02 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Robertson | 0.13 | 0.12 | 0.03 | 0 | 0.00 | 0.00 | 0.15 | 0.13 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.00 |
| **WCVI** | **0.26** | **0.22** | **0.03** | **0** | **0.00** | **0.01** | **0.15** | **0.13** | **0.01** | **0** | **0.00** | **0.01** | **0** | **0.00** | **0.01** |
| Quinsam | 0.17 | 0.15 | 0.05 | 0 | 0.02 | 0.03 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.03 | 0.04 |
| Big Qualic | 0.26 | 0.22 | 0.04 | 0.17 | 0.14 | 0.03 | 0 | 0.01 | 0.02 | 0 | 0.01 | 0.01 | 0.25 | 0.19 | 0.04 |
| **ECVI** | **0.43** | **0.37** | **0.06** | **0.17** | **0.16** | **0.04** | **0** | **0.02** | **0.02** | **0** | **0.02** | **0.02** | **0.25** | **0.22** | **0.05** |
| Toboggan | 0 | 0.00 | 0.00 | 0.26 | 0.24 | 0.03 | 0 | 0.00 | 0.00 | 0.14 | 0.15 | 0.03 | 0 | 0.01 | 0.01 |
| Cedar | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.01 | 0.13 | 0.09 | 0.03 | 0.20 | 0.13 | 0.03 |
| Babine | 0 | 0.00 | 0.01 | 0.17 | 0.16 | 0.02 | 0 | 0.00 | 0.00 | 0.26 | 0.25 | 0.04 | 0 | 0.00 | 0.00 |
| Clearwater | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.00 | 0.17 | 0.14 | 0.03 | 0 | 0.01 | 0.01 |
| Sustut | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0.13 | 0.10 | 0.03 | 0 | 0.00 | 0.01 |
| **Skeena** | **0** | **0.01** | **0.02** | **0.43** | **0.42** | **0.03** | **0** | **0.00** | **0.00** | **0.83** | **0.73** | **0.04** | **0.20** | **0.15** | **0.03** |
| Tseax | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 |
| Zolzap | 0 | 0.00 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 |
| Meziadin | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0.17 | 0.13 | 0.03 | 0 | 0.01 | 0.01 |
| **Nass** | **0** | **0.02** | **0.03** | **0** | **0.02** | **0.02** | **0** | **0.01** | **0.00** | **0.17** | **0.15** | **0.03** | **0** | **0.03** | **0.02** |
| Coldwater | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.03 | 0.02 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Salmon | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.00 | 0.10 | 0.09 | 0.01 |
| Eagle | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Spius | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0.20 | 0.16 | 0.03 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Lemieux | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Dunn | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Louis | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| Deadman | 0 | 0.01 | 0.01 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.01 | 0.00 |
| Bridge | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 | 0 | 0.00 | 0.00 |
| **TR** | **0** | **0.02** | **0.01** | **0** | **0.01** | **0.01** | **0.20** | **0.21** | **0.01** | **0** | **0.01** | **0.01** | **0.10** | **0.10** | **0.01** |
| Chilliwack | 0 | 0.04 | 0.03 | 0 | 0.01 | 0.01 | 0.10 | 0.10 | 0.04 | 0 | 0.00 | 0.01 | 0 | 0.02 | 0.03 |
| Chehalis | 0 | 0.01 | 0.02 | 0 | 0.01 | 0.02 | 0.25 | 0.18 | 0.03 | 0 | 0.01 | 0.01 | 0 | 0.02 | 0.03 |
| Stave | 0 | 0.01 | 0.02 | 0 | 0.02 | 0.02 | 0 | 0.02 | 0.02 | 0 | 0.01 | 0.02 | 0 | 0.03 | 0.03 |
| Upper Pitt | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.01 | 0 | 0.01 | 0.02 | 0 | 0.00 | 0.01 | 0.10 | 0.08 | 0.04 |
| Nicomen | 0 | 0.01 | 0.02 | 0 | 0.01 | 0.01 | 0.10 | 0.10 | 0.03 | 0 | 0.00 | 0.01 | 0 | 0.01 | 0.02 |
| Norrish | 0 | 0.01 | 0.02 | 0 | 0.01 | 0.02 | 0 | 0.02 | 0.02 | 0 | 0.01 | 0.02 | 0 | 0.03 | 0.03 |
| Inch | 0 | 0.01 | 0.01 | 0.13 | 0.09 | 0.03 | 0.20 | 0.17 | 0.03 | 0 | 0.00 | 0.01 | 0.15 | 0.11 | 0.03 |
| **L Fr** | **0** | **0.10** | **0.04** | **0.13** | **0.16** | **0.03** | **0.65** | **0.60** | **0.03** | **0** | **0.04** | **0.03** | **0.25** | **0.30** | **0.05** |

of our study on the basis of only three microsatellite loci, albeit ones selected for high levels of intra- and interpopulation polymorphism. The four major regional components of diversity in B.C. coho salmon almost certainly constitute the phylogeographic legacy of the last ice age, which ended 10–15,000 years ago. When the headwaters of the neighboring Columbia River system were in contact with the upper reaches of the Thompson-Fraser watershed, Columbia River coho salmon recolonized the Thompson and upper Fraser River (McPhail and Lindsey, 1986; Wehrhahn and Powell, 1987; Small et al., 1998). Coho salmon from the northern Bering Sea-Yukon River glacial refuge dispersed southward by

**Table 6**

Percentage of classifications of individual fish to regions for 34 populations of coho salmon from British Columbia. In this jackknife analysis the fish tested was not included in the discriminant analysis sample, which included the rest of the fish. The percentage of fish correctly classified to region is in the "correct" column and the percentage of fish from that region misclassified to other regions is read across the row. The percentage of fish from other regions misclassified to a particular region is read down the region column. *n* is the number of fish from the region.

| | | Region | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | *n* | Correct | Pallant | C. Coast | L. Fraser | Thompson | WCVI | ECVI | NCVI | Nass | Skeena | Total |
| Pallant | 92 | 48.91 | | 6.52 | 8.70 | 5.43 | 6.52 | 7.61 | 13.04 | 1.09 | 2.17 | 100 |
| C. Coast | 219 | 27.85 | 10.05 | | 5.94 | 6.85 | 4.11 | 17.81 | 11.42 | 7.31 | 8.68 | 100 |
| L. Fraser | 1018 | 53.73 | 4.72 | 5.89 | | 2.85 | 3.05 | 8.74 | 12.87 | 5.11 | 3.05 | 100 |
| Thompson | 798 | 84.96 | 1.13 | 2.51 | 2.63 | | 0.25 | 3.26 | 3.01 | 1.00 | 1.25 | 100 |
| WCVI | 115 | 60.00 | 6.09 | 6.09 | 11.30 | 2.61 | | 5.22 | 3.48 | 2.61 | 2.61 | 100 |
| ECVI | 338 | 44.08 | 5.03 | 10.36 | 13.61 | 2.37 | 5.03 | | 10.06 | 6.80 | 2.66 | 100 |
| NCVI | 133 | 43.61 | 4.51 | 5.26 | 18.05 | 6.02 | 1.50 | 10.53 | | 6.77 | 3.76 | 100 |
| Nass | 133 | 20.30 | 0.75 | 13.53 | 14.29 | 6.02 | 6.77 | 8.27 | 9.02 | | 21.05 | 100 |
| Skeena | 412 | 52.67 | 3.40 | 5.58 | 6.55 | 6.07 | 1.94 | 6.80 | 6.31 | 10.68 | | 100 |

sea, or traversed the shifting freshwater waterways of northern B.C., to recolonize the upper reaches of major watersheds as far south as the Nass and Skeena rivers (Lindsey and McPhail, 1986). Dispersal of coho salmon from a central B.C. coastal refuge(ia) located on the mainland or unglaciated portions of Vancouver or the Queen Charlotte Islands (or both) (Warner et al., 1982) likely established the heterogeneous mainland-coastal population group, of which lower Fraser coho salmon populations may be a distinctive offshoot.

Of the four regional components of biodiversity in B.C. coho salmon, the Thompson and upper Fraser is the most distinctive. Little introgression has apparently occurred between the lower Fraser and Thompson River coho salmon populations despite their common passage through the lower Fraser River on their return to spawning grounds for over 3,000 generations. Coho salmon populations are few and small in the Fraser River drainage above its confluence with the Thompson River, and our data show no evidence of introgression between the coho salmon of the Thompson-Fraser Rivers and the upper Skeena watersheds such as that postulated for sockeye salmon (Wood et al., 1994). However, our sampling of the Skeena watershed is limited to date, and the current analysis (in which larger, relatively infrequent, alleles have been binned) has limited power for the detection of historical gene exchange through an analysis of rare alleles.

The Skeena River watershed has been identified as the southern limit for freshwater fish and sockeye salmon that dispersed from a northern glacial refuge (Lindsey and McPhail, 1986; Wood et al., 1994; Bickham et al., 1995). Thus, it seems likely that the coho salmon populations of the upper Skeena and Nass watersheds are derived from Beringia. The genetic intermediacy of lower Skeena and Nass populations between upper Skeena-Nass and neighboring coastal populations suggests a hybrid nature for the lower river populations. The extent to which the composite nature of these populations reflects historical or current gene flow (or both) between the two founding groups, and the adaptive consequences of such introgression, has yet to be determined.

The coho populations of the lower Fraser drainage basin formed a cohesive genetic group in the NJ analysis of genetic distance in this study. This was in sharp contrast to the heterogeneity observed among other coastal mainland and island populations likely derived from one or more coastal refugia. The heterogeneity of central coast populations may reflect the existence of several coastal refugia, introgression from northern coho populations originating from Beringia that have yet to be well characterized, or simply founder effects in the establishment of individual coastal populations from a single refuge, as postulated for sockeye salmon (Wood et al., 1994). If the heterogeneity does result from an admixture of several founding groups among coastal coho populations, the lower Fraser River populations may better represent the original genetic profile of fish from a single, possibly southern, coastal refuge. Interestingly, Vancouver Island coho salmon were more frequently misclassified as lower Fraser than as central coast fish in the mixed-stock fishery simulations of our study, in spite of their apparently greater genetic similarity to central coast populations. If the

lower Fraser River populations are characteristic of coho salmon derived from a refuge located on, or to the south of, Vancouver Island, Puget Sound coho salmon populations might be expected to be of similar origin. This is consistent with the inclusion, based primarily on genetic data, of lower Fraser and southeastern Vancouver Island coho salmon populations in a Puget Sound and Strait of Georgia ESU (Weitkamp et al., 1995). Further sampling of Vancouver Island populations will be necessary to define the boundaries of the historical groups of coho salmon that likely converge there.

The geographic basis for population structure of coho salmon revealed in this study is remarkably similar to that described for sockeye salmon based on allozyme and mtDNA data (Wood et al., 1994; Bickham et al., 1995). A genetic discontinuity between chinook salmon originating from the Columbian and Beringial glacial refugia (Gharrett et al., 1987; Cronin et al., 1993) also lies in British Columbia, and dispersal from a coastal refuge(ia) can be traced in the microsatellite data for chinook salmon as well (Beacham, unpubl. data). Thus, the phylogeographic reconstruction of postglacial dispersal based on freshwater fish distributions in British Columbia (McPhail and Lindsey, 1970, 1986; Lindsey and McPhail, 1986) has proven to be taxonomically robust and also provides the foundation for the genetic architecture of anadromous Pacific salmon species.

The mixed-stock fishery analyses demonstrated the utility of microsatellite DNA variation for coho salmon stock identification. We obtained accurate and precise estimates both of population contributions in mixed-stock samples from a single drainage and of population and regional contributions in mixed-stock samples drawn from several regions. An important feature of the microsatellite data set is the strong regional structuring of the observed genetic variation, which means that contributions from populations present in a mixture sample, but not in the baseline, will be identified correctly to region. Identifying individual fish to correct populations is more difficult than estimating percentage contributions to a stock mixture, because only characteristics of individual fish are used in the classification. In general, the microsatellite loci of this study provided a similar level of accuracy in the classification of individual coho salmon to population and region as did minisatellite DNA markers (Beacham et al., 1996). Within the Fraser River drainage, identification of individual fish was more accurate with microsatellite data (correct identification of 54% of lower Fraser and 85% of upper Fraser Rive coho salmon) than with minisatellite data (correct identification of 30% and 60% of the respective groups). The identification of individual fish is an important enforcement tool, and may improve as more microsatellite loci are added to the database.

The results of this study are consistent with a depiction of population structure in coho salmon as distinct phylogenetic lines composed of geographically based metapopulations (McPhail, 1997). The more consistent regional grouping of coho salmon populations than of sockeye salmon (Wood et al., 1994) may reflect greater, or more recent, gene flow among geographically proximate coho salmon populations than among similar sockeye populations, or may reflect differences between allozyme- and microsatellite-based data sets. Moreover, the increasing power of genetic methods applied to coho salmon data, as demonstrated in this and other (Weitkamp et al., 1995; Beacham et al., 1996; Van Doornik et al., 1996; Miller et al., 1996; Miller and Withler, 1997; Small et al., 1998) studies, enable us not only to delineate regional (metapopulation) structure in coho salmon but also to identify the regional contributions of coho from different metapopulations in mixed-stock fishery harvests. Thus, the challenge for metapopulation-based coho salmon management may lie not so much in the delineation of metapopulation structure (McPhail, 1997) as in the evaluation of the biological and social costs associated with the loss, even if only temporary in historical terms, of the less productive components of metapopulation structure during periods of overall low abundance.

Additional aspects of metapopulation theory, as applied to Pacific salmonids, need to be addressed before this model of population structure will support practical management decisions. Managers need to know, at any given point in history, how the adaptive genetic diversity of a metapopulation is likely to be distributed among its subpopulations, and how many subpopulations can be lost before evolutionary potential is compromised. This depends on, among other things, which model of metapopulation structure is adopted. Are salmonid metapopulations of the "source-sink" variety in which gene flow is basically unidirectional from large source subpopulations to ephemeral sink subpopulations (Pulliam, 1988; Pulliam and Danielson 1991)? Or are salmonid metapopulations of the "balanced exchange" type, in which gene flow is bidirectional and migration rates are inversely proportional to subpopulation size (McPeek and Holt, 1992; Doncaster et al., 1997)? Current models of metapopulation structure have been more extensively investigated with respect to population dynamics (extinctions and recolonizations among subpopulations) than with respect to population and evolutionary genetics (the spatial and tem-

poral distribution of genetic diversity among subpopulations). The finding in this, and other molecular genetic studies on coho salmon (Beacham et al., 1996; Miller et al., 1996), of temporally stable allele frequency differences at neutral loci among local "stocks" (subpopulations in the metapopulation model) may indicate that there is very little effective gene flow among subpopulations (and few recolonization events in vacant habitat) on time scales of relevance to managers.

In summary, we have demonstrated that variation at microsatellite loci can be used both to define the regional and local components of coho salmon population structure in British Columbia and to identify these elements in stock composition estimation for mixed-stock fishery analysis. Molecular genetic technology will enable delineation of metapopulations or ESUs (or both) in the coho salmon of British Columbia, but management tools based on these concepts are lacking.

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## Literature cited

**Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers.**
**1995**. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biol. 47, (suppl. A): 177–185.

**Bartley, D. M., B. Bentley, P. G. Olin, and G. A. E. Gall.**
**1992.** Population genetic structure of coho salmon (*Oncorhynchus kisutch*) in California. Calif. Fish and Game 78:88–104.

**Beacham, T. D., L. Margolis, and R. J. Nelson.**
**1998**. A comparison of methods of stock identification for sockeye salmon (*Oncorhynchus nerka*) in Barkley Sound, British Columbia. N. Pac. Andr. Fish Comm. Bull. 1: 227–239.

**Beacham, T. D., K. M. Miller, and R. E. Withler.**
**1996**. Minisatellite DNA variation and stock identification of coho salmon. J. Fish Biol. 48:1–19.

**Beamish, R. J., and D. R. Bouillon.**
**1993.** Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci. 50:1002–1016.

**Bickham, J. W., C. C. Wood, and J. C. Patton.**
**1995.** Biogeographic implications of cytochrome *b* sequences and allozymes in sockeye (*Oncorhynchus nerka*). J. Hered. 86:140–144.

**Cavalli-Sforza, L. L., and A. W. F. Edwards.**
**1967**. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Gen. 19:233–257.

**Cronin, M. A., W. J. Spearman, R. L. Wilmot, J. C. Patton, and J. W. Bickham.**
**1993.** Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. Can. J. Fish. Aquat. Sci. 50:708–715.

**Dempster, A. P., N. M. Laird, and D. B. Rubin.**
**1977**. Maximum likelihood from incomplete data via the EM algorithm. J. Royal Stat. Soc. B 39:1–38.

**Doncaster, C. P., J. Clobert, B. Doligez, L. Gustafsson, and E. Danchin.**
**1997.** Balanced dispersal between spatially varying local populations: an alternative to the source-sink model. Am. Nat. 150:425–445.

**Felsenstein, J.**
**1993**. PHYLIP, version 3.4. Department of Genetics, Univ. Washington, Seattle, WA, unpaginated.

**Fraser, F. J., P. J. Starr, and A. Y. Federenko.**
**1982.** A review of the chinook and coho salmon of the Fraser River. Can. Tech. Rep. Fish. Aquat. Sci. 1126, 130 p.

**Forbes, S., K. Knudsen, and F. Allendorf.**
**1993.** Genetic variation in DNA of coho salmon from the Lower Columbia River. Final Report to the U.S. Dep. Energy, BPA., Div. Fish and Wildlife, Contract DE-BI179-92BP 30198, 25 p.

**Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack.**
**1984**. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41:400–408.

**Galbraith, D. A., P. T. Boag, H. L. Gibbs, and B. N. White.**
**1991**. Sizing bands on autoradiograms: a study of precision for scoring DNA fingerprints. Electrophoresis 12:210–220.

**Gharrett, A. J., S. M. Shirley, and G. R. Tromble.**
**1987.** Genetic relationships among populations of Alaskan chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 44:765–774.

**Gill, P., K. Sullivan, and D. J. Werrett.**
**1990**. The analysis of hypervariable DNA profiles: problems associated with the objective determination of the probability of a match. Hum. Genet. 85:75–79.

**Goudet, J.**
**1995.** Fstat, version 1.2: a computer program to calculate *F*-statistics. J. Hered. 86:485–486.

**Guo S. W., and E. A. Thompson.**
**1992.** Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361–372.

**Lessios, H. A.**
**1992**. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Mar. Biol. 112:517–523.

**Lindsey, C. C., and J. D. McPhail.**
**1986.** Zoogeography of fishes of the Yukon and Mackenzie basins. *In* C. H. Hocutt and E. O. Wiley (eds.), Zoogeography of North American freshwater fishes, p. 639–674. John Wiley & Sons, Inc., New York, NY.

**McConnell, S. K., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen.**
**1995**. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American

and European populations. Can. J. Fish. Aquat. Sci. 52:1863–1872.

**McPeek, M. A., and R. D. Holt.**
**1992.** The evolution of dispersal in spatially and temporally varying environments. Am. Nat. 140:1010–1027.

**McPhail, J. D.**
**1997.** The origin and speciation of *Oncorhynchus* revisited. *In* D. J. Stouder, P. A. Bisson and R. J. Naiman (eds.), Pacific salmon and their ecosystems: status and future options, p. 29–38. Chapman and Hall, New York, NY.

**McPhail, J. D., and C. C. Lindsey.**
**1970.** Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173, 381 p.
**1986.** Zoogeography of freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). *In* C. H. Hocutt and E. O. Wiley (eds.), Zoogeography of North American freshwater fishes, p. 615–637. John Wiley & Sons, Inc., New York, NY.

**Miller, K. M., and R. E. Withler.**
**1997.** *Mhc* diversity in Pacific salmon: population structure and trans-species allelism. Hereditas 127:83–95.

**Miller, K. M., R. E. Withler, and T. D. Beacham.**
**1996.** Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation. Can. J. Fish. Aquat. Sci. 53:181–195.

**Nehlsen, W., Williams, J. E., and J. A. Lichatowich.**
**1991.** Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 16(2):4–21.

**Northcote, T. G., and D. Y. Atagi.**
**1997.** Pacific salmon abundance trends in the Fraser river watershed compared with other British Columbia systems. *In* D. J. Stouder, P. A. Bisson, and R. J. Naiman (eds.), Pacific salmon and their ecosystems: status and future options, p. 199–219. Chapman and Hall, New York, NY.

**Pulliam, H. R.**
**1988.** Sources, sinks, and population regulation. Am. Nat. 132:652–661.

**Pulliam, H. R., and B. J. Danielson.**
**1991.** Sources, sinks, and habitat selection—a landscape perspective on population dynamics. Am. Nat. 137:S50–S66.

**Raymond, M., and F. Rousset**
**1995a.** GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. Hereditary 86:248–249.
**1995b.** An exact test for stock differentiation. Evolution 49:1280–1283.

**Reisenbichler, R. R.**
**1997.** Genetic factors contributing to declines of anadromous salmonids in the Pacific northwest. *In* D. J. Stouder, P. A. Bisson, and R. J. Naiman (eds.), Pacific salmon and their ecosystems: status and future options, p. 223–224. Chapman and Hall, New York, NY.

**Ricker, W. E.**
**1972.** Heredity and environmental factors affecting certain salmonid populations. *In* R. C. Simon and P. A. Larkin (eds.), The stock concept of Pacific salmon, p. 19–60. H. R. MacMillan lectures in fisheries, Univ. British Columbia, Vancouver.

**Roff, D. A., and P. Bentzen.**
**1989.** The statistical analysis of mitochondrial DNA polymorphisms: $\chi^2$ and the problem of small sample sizes. Mol. Biol. Evol. 6:539–545.

**Saitou, N., and M. Nei.**
**1987.** The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

**Sakai, R. K., S. Scharf, F. Falloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.**
**1985.** Enzymatic amplification of Beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science (Wash., D.C.) 230:1350–1354.

**SAS Institute Inc.**
**1989.** SAS/STAT user's guide, version 6, 4th edition. vol. 1. p. 678–771. SAS Institute Inc., Cary, NC.

**Scribner, K. T., J. R. Gust, and R. L. Fields.**
**1996.** Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can. J. Fish. Aquat. Sci. 53:833–841.

**Small, M. P., T. D. Beacham, R. E. Withler, and R. J. Nelson.**
**1998.** Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia. Mol. Ecol. 7:141–155.

**Tautz, D.**
**1989.** Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nuc. Acid Res. 17:6563–6471.

**Van Doornik, D. M., G. B. Milner, and G. A. Winans.**
**1996.** Transferrin polymorphism in coho salmon, *Oncorhynchus kisutch*, and its application to genetic stock identification. Fish. Bull. 94:566–575.

**Walters, C. J.**
**1993.** Where have all the coho gone? *In* L. Berg and P. W. Delaney (eds.), Proceedings of the coho salmon workshop, Nanaimo, B.C., May 26–28, 1992, p. 1–8. Department of Fisheries and Oceans, Nanaimo.

**Waples, R. S.**
**1991.** Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Mar. Fish. Rev. 53(3):11–22.

**Warner, B. G., R. W. Mathewes, and J. J. Clague.**
**1982.** Ice-free conditions on the Queen Charlotte Islands, British Columbia, at the height of late Wisconsin glaciation. Science (Wash. D.C.) 218:675–677.

**Wehrhahn, C. F., and R. Powell.**
**1987.** Electrophoretic variation, regional differences and gene flow in the coho salmon (*Oncorhynchus kisutch*) of Southern British Columbia. Can. J. Fish. Aquat. Sci. 44:822–831.

**Weir, B. S., and C. C. Cockerham.**
**1984.** Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

**Weitkamp, L. A., T. C. Wainwright, G. J. Bryant, G. B. Milner, D. J. Teel, R. G. Kope, and R. S. Waples.**
**1995.** Status review of coho salmon from Washington, Oregon, and California. U.S. Dep. Commerce, NOAA Tech. Memo. NMFS-NWFSC-24, 258 p.

**Wood, C. C., B. E. Riddell, D. T. Rutherford, and R. E. Withler.**
**1994.** Biochemical genetic survey of sockeye salmon (*Oncorhynchus nerka*) in Canada. Can. J. Fish. Aquat. Sci. 51:114–131.

# Gonadal development and associated changes in plasma reproductive steroids in English sole, *Pleuronectes vetulus*, from Puget Sound, Washington

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**Abstract.**—Gonadal development, and associated changes in reproductive parameters were investigated in adult female and male English sole *(Pleuronectes vetulus)* in Puget Sound to provide baseline data for future research on effects of contaminants on reproductive endocrine function in this species. Changes in gonadal histology, gonadosomatic index (GSI), and plasma reproductive steroids (female: 17β-estradiol [E2], testosterone [T]; male: T, 11-ketotestosterone [11KT]) were monitored throughout the spawning season. Female sole sampled July through early September were primarily regressed or previtellogenic and had low GSI and plasma steroid levels. GSI and plasma steroid levels were also low in male sole sampled during this time, but the majority had already entered spermatogenesis and, in some fish, production of mature sperm was observed. Fish sampled in October were in the early stages of vitellogenesis and spermiogenesis and showed increases in GSI and plasma steroid levels. By November, about 50% of female fish had entered vitellogenesis and about 30% of males were producing sperm. Proportions of vitellogenic females and sperm-producing males continued to increase through January, with significant numbers of spawning females and males present in February. By late March, the majority of both sexes were spent. Vitellogenic female sole had the highest plasma E2 levels, vitellogenic sole with hydrated oocytes had the highest GSI, and spawning female sole had the highest plasma T levels. Plasma T, 11KT, and GSI were highest in spawning male sole. Reproductive parameters returned to baseline levels in spawned out female and male sole. A potent maturation-inducing steroid (MIS) in many species of teleosts, 17α, 20β-dihydroxy-4-pregnen-3-one, was not detected in spawning English sole. Additional research is needed to identify the MIS in English sole and to understand better the hormonal regulation of early gonadal development in male sole.

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English sole *(Pleuronectes vetulus)* is a commercially important flatfish species indigenous to the west coast of North America. It is also a primary sentinel species for a number of environmental monitoring programs on the west coast of the United States, including the National Benthic Surveillance Project (Myers et al., 1994) and the Puget Sound Ambient Monitoring Program (PSWQA[1]). Studies suggest that female English sole are quite sensitive to environmental contaminants. For example, female sole from several sites within Puget Sound, WA, with high levels of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (in sediment), exhibit various types of reproductive dysfunction, including depressed sex hormone levels, altered or inhibited reproductive development, and reduced egg and larval viability (Johnson et al., 1988, 1993; Casillas et al., 1991; Collier et al., in press).

Male English sole may also be at risk for reproductive injury. Recent studies suggest that exposure to certain chemical compounds (e.g. alkylphenols, phthalates, and some PCBs and chlorinated pesticides) can reduce testicular growth and sperm production in male teleosts (Jobling et al., 1996; Nimrod and Benson, 1996), and alterations in reproductive steroids have been reported in male fish exposed to oil and other pollutants (Truscott et al., 1992a; Idler et al., 1995). However, no comprehensive studies have been conducted on the effects of environmental contaminants on reproductive function in male English sole. Before such studies can be carried out effectively, the normal reproductive endocrine cycle of male English sole must be characterized.

The general life history of English sole is well known (Ketchen, 1947; Harry, 1959; Garrison and Miller, 1982), and its cycle of oocyte development and related changes in estradiol-17β (E2) and other plasma parameters have been described

[1] PSWQA (Puget Sound Water Quality Authority). 1995. 1994 Puget Sound update: fifth annual report of the Puget Sound Ambient Monitoring Program. Puget Sound Water Quality Authority, Olympia, WA, 122 p.

(Johnson and Casillas, 1991; Fargo and Tyler, 1994). However, no information exists on other reproductive steroids involved in oocyte development and spawning in female English sole, or on changes in reproductive parameters in male English sole during gonadal recrudescence. This study was designed to gain a better understanding of reproductive processes in both female and male English sole. This information can be used to assess the impact of environmental degradation on their reproductive development and to develop effective techniques for flatfish mariculture. It may also be useful in evaluating the reproductive status of English sole stocks that are a fisheries resource off the northwest coast of the United States.

Wild populations of female and male English sole were sampled during the 1992–93 and 1994–95 spawning cycles at two residential sites as well as at a known spawning ground in Puget Sound, Washington. Several reproductive parameters, including gonadosomatic index (GSI), histologically determined stages of gonadal development, and steroid hormones (female: plasma testosterone [T] and E2; male: T and 11-ketotestosterone [11KT]) were monitored throughout the spawning season. Additionally, 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-P), a potent maturation inducing steroid (MIS) in many teleosts (see review by Scott and Canario, 1987), was tested as the potential MIS in English sole.

## Materials and methods

### Chemicals

Tritium-labeled steroids were purchased from DuPont NEN (USA), and Amersham (UK). The antibodies for E2 and T were purchased from G. Niswender (Colorado State University, United States). The 11KT antibody was a gift from Y. Nagahama (Japan), and the 17α, 20β-P antibody was a gift from A. P. Scott (United Kingdom). The standards for the steroids were purchased from Steraloids, Inc. (USA).

### Collection of samples

The sampling times for fish were chosen to coincide with the period of gonadal recrudescence and spawning in English sole (Ketchen, 1947; Harry, 1959; Johnson et al., 1991). Maturing female (>200 mm total length (TL)) and male sole ( >190 mm TL) were collected from Tulalip Bay (October through January) and University Point (January through March) by otter trawl during the 1992–93 (October–March) and 1993–94 (October) spawning cycles. Additional sole were collected monthly at Pilot Point from February 1994 to December 1995. Tulalip Bay and Pilot Point (residential sites) were chosen for their large populations of English sole, and University Point was chosen because sole migrate to this area for spawning (Johnson et al., 1991; Collier et al., 1992).

Fish were kept in holding tanks aboard the research vessel for approximately one hour before sampling was carried out. The sole were weighed (to the nearest g) and measured (to the nearest mm), and blood (1 mL) was collected from the caudal vein with a heparinized syringe. Blood samples were centrifuged for 10 minutes at 800 × g, and plasma was collected and stored in triplicate bullet vials (250 µL each vial) for subsequent analysis of reproductive steroids (females: E2, T, and 17α, 20β-P; males: 11KT, and T). Immediately following blood collection, the fish were sacrificed by severing the spinal cord. The gonads were removed and weighed, and samples of gonadal tissue were collected and preserved in Dietrich's fluid (Gray, 1954) for histological examination. The abdominal contents were removed and the gutted carcass was weighed. Plasma samples were frozen and stored at –80°C until steroid analyses could be performed.

### Sample analyses

Plasma T, E2, and 11KT levels were determined by radioimmunoassay (RIA) as described by Sower and Schreck (1982), and 17α, 20β-P levels were determined by RIA as described by Scott et al. (1982). Each RIA (E2, 11KT, and T, and 17α, 20β-P) consisted of duplicates of standards (ranging from 0 to 2.0 ng/mL), samples, and quality control samples (pooled plasma from male or female English sole). Detection limits of the assays (20–80% binding range of the standard curve) were 0.006–0.3 ng/mL plasma for E2; 0.009–0.4 ng/mL for 11KT; 0.002–0.13 ng/mL for T; and 0.003–0.08 ng/mL plasma for 17α, 20β-P. For each assay, samples with steroid concentrations outside the detection limits of the assay were retested by adjusting the sample volume. All samples were corrected for dilution factor. Mean (±SE) of quality control samples were 4.4 ±0.55 ng/mL for E2 ($n$=15); 0.89 ±0.12 ng/mL for 11KT ($n$=6); and 0.29 ±0.04 ng/mL for T ($n$=16). The steroid 17α, 20β-P was not detected in quality control samples.

Gonadal tissues collected for histological examination were embedded in paraffin, sectioned, stained with Harris hematoxylin and eosin-phloxine (Luna, 1968) and examined by light microscopy. Ovarian developmental stage (Table 1) was classified accord-

**Table 1**

Classification scheme for female ovarian developmental stages, modified from histological criteria outlined in Johnson et al. (1991).

| Stage | Features |
|---|---|
| Regressed | Primary and secondary oocytes |
| Previtellogenic | Vacuolated secondary oocytes with clear peripheral vacuoles; zonal radiata present |
| Vitellogenic | Yolked oocytes present |
| Vitellogenic with hydrated oocytes | Yolked globules coalescing; hydrated oocytes present, no postovulatory follicles (POFs) |
| Spawning | Hydrated oocytes with POFs |
| Spawned out | Many POFs visible, yolked oocytes undergoing resorption, and inflammatory infiltrate, beta or gamma atretic follicles or macrophage aggregates (or both) generally present |

ing to the criteria modified from Johnson et al. (1991). Testicular developmental stage was classified as modified from Billard (1992). GSI was calculated by using the formula GSI = (gonad weight (g) /gutted weight (g)) × 100.

### Statistical analysis

Mean (±SE) GSI and plasma reproductive steroid levels were calculated by month and gonadal maturation stage. Analysis of variance (ANOVA) and subsequent multiple comparison testing by Fisher's protected least significant difference (PLSD) test (95% CI), were used to test for changes in these factors during the reproductive cycle (Dowdy and Wearden, 1991). The data was normalized by log transformation prior to statistical comparisons.

## Results

### Classification of testis

The testicular developmental stages for male English sole are shown in Figure 1, A–F. The testis consists of tubules of reproductive cells in various stages of development. Testicular development is categorized into six stages: regressed (Fig. 1A), early recrudescence (Fig. 1B), late recrudescence (Fig. 1C), early spermiogenesis (Fig. 1D), spawning (Fig. 1E), and spawned out (Fig. 1F). Each stage is characterized as follows: in the regressed stage, tubules contain only spermatogonia; in early recrudescence, primary and secondary spermatocytes begin to appear; in the late recrudescent stage spermatogonia, spermatocytes (primary and secondary), and spermatids are evident, but no spermatozoa; in early spermiogenesis primarily spermatocytes (primary and secondary), spermatids, and early sperm production are evident; in the spawning stage predominately mature sperm fill the tubular space and sperm ducts; in the spawned-out testis, the tubular space and sperm ducts are largely empty and few sperm remain.

### Size at sexual maturation

The size of English sole captured in this study ranged from 212 to 455 mm for females and from 195 to 345 mm total length for males. Histological analyses of the gonads showed that a majority of the female sole <260 mm TL were immature (regressed–previtellogenic), and failed to reach vitellogenesis (Table 2). Male sole <230 mm reached spermatogenesis but most did not fully mature and spawn (Table 3). Female sole <260 mm were excluded from further statistical analyses, but no size limit was used for male sole.

### Proportions of fish undergoing gonadal maturation and spawning

**Female** Figure 2 shows the proportion of female sole at different ovarian developmental stages by month. Even among adult sole (>260 mm), regressed females were found all year, and the mean proportion of the regressed animals each month was 24% (±11%, SD). Developing females were found as early as July; 14% of the female sole sampled in July were vitellogenic. The proportion of vitellogenic sole generally increased until January (78%), then decreased in the following months; by March only 11% of the sole sampled were vitellogenic. Spawned out females were found as early as October (1%), and the proportion increased through March (31%).

**Male** Figure 3 shows the proportion of male sole at different testicular developmental stages categorized by month of capture. Animals collected in July consisted of regressed males (12%) and early developing males (25% each were at early and late recrudescence), and males in early spermiogenesis (38%). Spawning males were found as early as October (16%), and the proportion generally increased until February (91%). By March, 41% were spawning and 36% were spawned out. Unlike females, in which a certain proportion of fish did not develop during the reproductive cycle, almost all males collected did

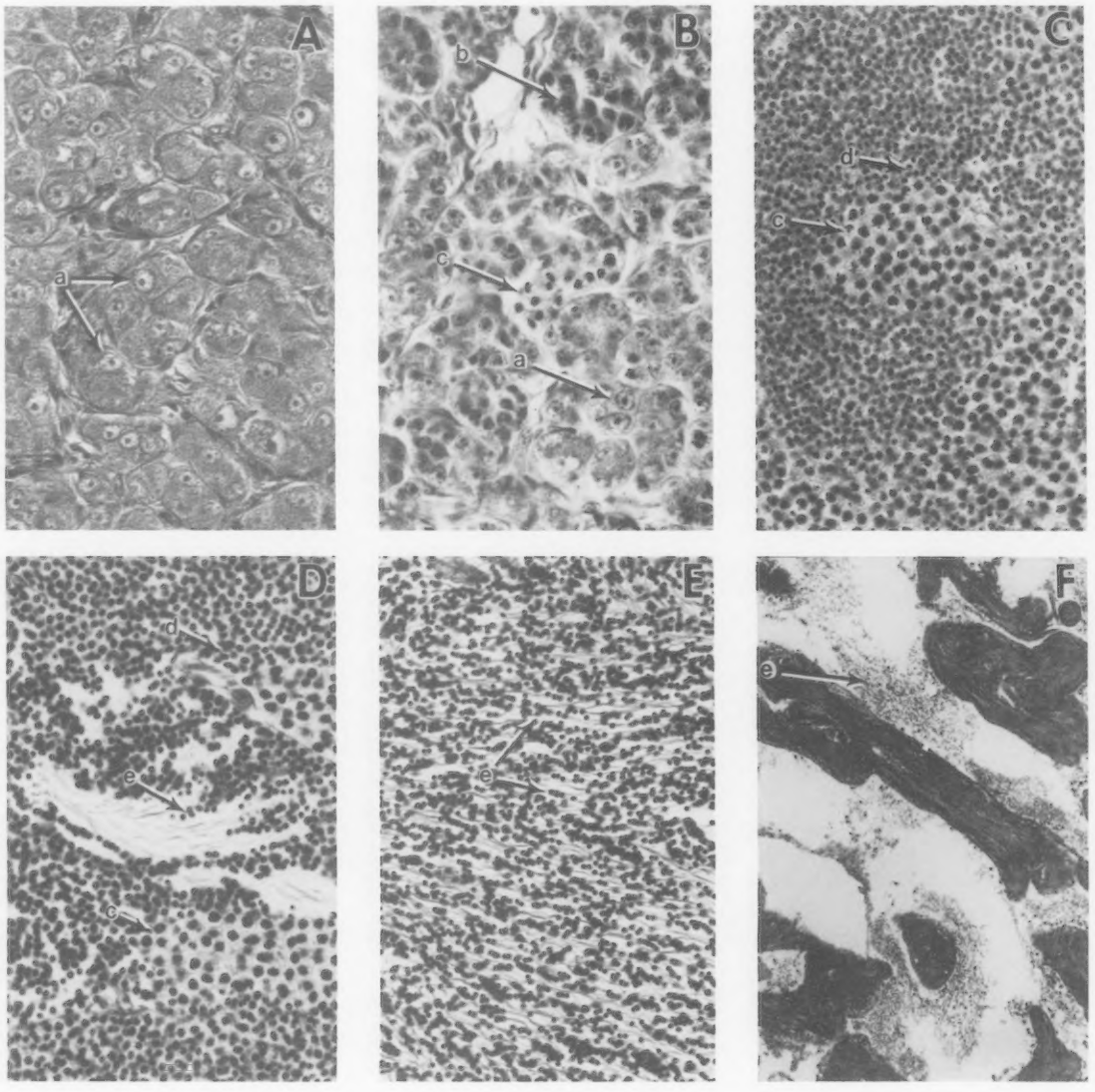


**Figure 1**

Micrographs of testicular developmental stages in male English sole: (**A**) regressed (510×), (**B**) early recrudescence (560×), (**C**) late recrudescence (560×), (**D**) early spermiogenesis (560×), (**E**) spawning (540×), and (**F**) spawned out (180×). a=spermatogonia, b=primary spermatocytes, c=secondary spermatocytes, d=spermatids, and e=spermatozoa.

enter spermiogenesis. However, only a small proportion of males <230 mm were actually producing sperm, and none in this size range were collected on the spawning ground. The smallest male caught at University Point measured 238 mm, suggesting that males <230 mm may not migrate to the spawning ground (University Point) (Table 3).

## Changes in reproductive parameters during gonadal recrudescence

**Female** Figure 4, A and B, shows reproductive parameters in female sole at each developmental stage. Generally, the immature (regressed and previtellogenic) sole had low GSI (1.4 ±0.1, $n$=85), and low

**Table 2**

Length maturation relationship in female English sole collected during months of gonadal recrudescence, October–March. Numbers represent percentage of the animals in the length class at each ovarian stage.

| Length (mm) | Regressed | Previtellogenic | Vitellogenic | Vitellogenic with hydrated oocytes | Spawning | Spawned out | *n* |
|---|---|---|---|---|---|---|---|
| <220 | 50 | 50 | | | | | 2 |
| 221–230 | 100 | | | | | | 1 |
| 231–240 | 100 | | | | | | 11 |
| 241–250 | 78 | 11 | 11 | | | | 9 |
| 251–260 | 69 | 15 | 15 | | | | 13 |
| 261–270 | 31 | 23 | 31 | 7.7 | | 7.7 | 13 |
| 271–280 | 32 | 16 | 37 | | | 16 | 19 |
| 281–290 | 44 | 11 | 44 | | | | 18 |
| 291–300 | 25.7 | 23 | 40 | 2.9 | | 8.6 | 35 |
| 301–310 | 34 | 13 | 44 | | 3.1 | 6.3 | 32 |
| 311–320 | 33 | 12 | 45 | | 3 | 6.1 | 33 |
| >320 | 26.9 | 16 | 47 | 2.9 | 7 | 11 | 172 |

plasma reproductive steroid concentrations (E2: 650 ±110 pg/mL plasma, $n$=66; T: 80 ±12 pg/mL plasma, $n$=66). Increases in GSI and reproductive steroids were observed in animals at the previtellogenic stage, and peak levels of GSI (20.0 ±4.8, $n$=9) and plasma E2 levels (7000 ±4700, $n$=4) were found in vitellogenic sole with hydrated oocytes, whereas peak plasma T levels (2300 ±620, $n$=9) were found in spawning females. All reproductive parameters were reduced in spawned out sole (GSI: 2.8 ±0.5, $n$=31; E2: 280 ±70, $n$=21; T: 90 ±50, $n$=21). 17α, 20β-P was not detected in female sole.

Figure 5, A–C, shows the mean levels of reproductive parameters in female sole at each month of collection. The sole sampled in July had low GSI (2.14 ±0.45, $n$=23) and plasma reproductive steroid levels (E2: 710 ±160, $n$=26; T: 100±30, $n$=27), which remained low until the onset of vitellogenesis (September–October for the majority of fish). The highest GSI (12.0 ±2.9, $n$=10) and plasma steroid levels (E2: 12000 ±5000, $n$=3; T: 2400 ±1200, $n$=4) were observed in January. By March, GSI and plasma reproductive steroid levels were reduced to levels comparable to those found in sole sampled in July. In general, fish from Tulalip Bay and Pilot Point had similar levels of reproductive parameters, which were lower than levels in fish from the University Point. The fish at University Point were either in final maturation or were actively spawning, whereas fish from Tulalip Bay and Pilot Point were migrating to spawning areas to undergo final maturation.

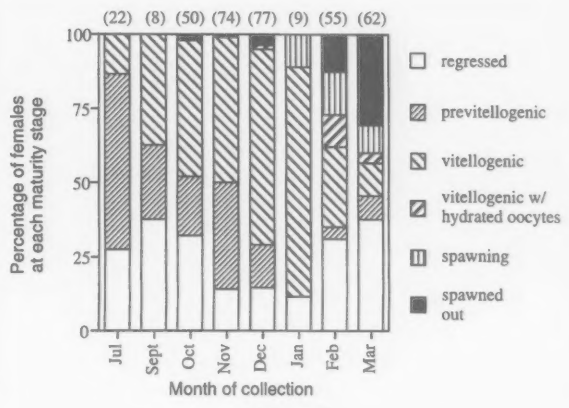


**Figure 2**

Percentage of female English sole at each stage of ovarian development by collection month. Numbers in parentheses represent animals sampled each month and histologically analyzed for ovarian stages.

**Male** Figure 6, A and B, shows the reproductive parameters in male sole at each developmental stage. The changes in reproductive parameters were similar to those observed in female sole. Generally, GSI (0.60 ±0.08, $n$=6) and plasma steroid levels (11KT: 1900 ±920 pg/mL plasma, $n$=4; T: 160 ±60 pg/mL plasma, $n$=4) were low in regressed animals. Increases in GSI and reproductive steroids were observed in animals at the early recrudescence stage and peaked in spawning fish (GSI: 1.6 ±0.1, $n$=91; 11KT: 5300 ±610, $n$=57; T: 830 ±130, $n$=64). Repro-

**Table 3**

Length maturation relationship in male English sole collected during months of gonadal recrudescence, October–March. Numbers represent percentage of the animals in the length class at each testicular stage.

| Length (mm) | Regressed | Early recrudescence | Late recrudescence | Early spermiogenesis | Spawning | Spawned out | *n* |
|---|---|---|---|---|---|---|---|
| <220 | | | | 85 | 15 | | 13 |
| 221–230 | 6.7 | 13 | 6.7 | 60 | 13 | | 15 |
| 231–240 | 13 | 6.7 | 13 | 33 | 27 | 6.7 | 15 |
| 241–250 | | 6.7 | 3.3 | 27 | 60 | 3.3 | 30 |
| 251–260 | 5.6 | 5.6 | 0 | 47 | 39 | 2.8 | 36 |
| 261–270 | | 2.9 | 2.9 | 49 | 40 | 5.7 | 35 |
| 271–280 | | 4.8 | | 43 | 48 | 4.8 | 21 |
| 281–290 | | 6.7 | | 40 | 53 | | 15 |
| 291–300 | | | | 36 | 64 | | 14 |
| 301–310 | | | | 38 | 50 | 13 | 8 |
| 311–320 | | | | 25 | 50 | 25 | 4 |
| >320 | 10 | | 10 | 10 | 70 | | 10 |

ductive parameters were reduced in spawned out fish (GSI: 0.8 ±0.1, $n$=8; 11KT: 83 ±5, $n$=8; T: 24 ±7, $n$=7), and plasma steroid levels in spawned out fish were significantly lower than levels observed in fish at other stages of testicular development.

Figure 7, A–C, shows the mean levels of reproductive parameters in male sole at each month of collection. GSI in sole sampled during July was low (0.89 ±0.18, $n$=6), increased to a peak in January (2.6 ±0.7, $n$=4), and returned to baseline levels in sole sampled in March (0.8 ±0.8, $n$=22). Plasma steroid levels were low in sole sampled in July (11KT: 890 ±140, $n$=13; T: 120 ±30, $n$=12) even though a few fish expressed milt. The levels increased to a peak in February (11KT: 13000 ±2800, $n$=6; T: 1800 ±340, $n$=14), and decreased in March (11KT: 1300 ±950, $n$=18; T: 400 ±310, $n$=15). Highest levels of plasma T and 11KT were found in fish from University Point where fish had migrated to spawn. A few fish with comparable levels of 11KT were found in March from the residential site at Tulalip Bay.

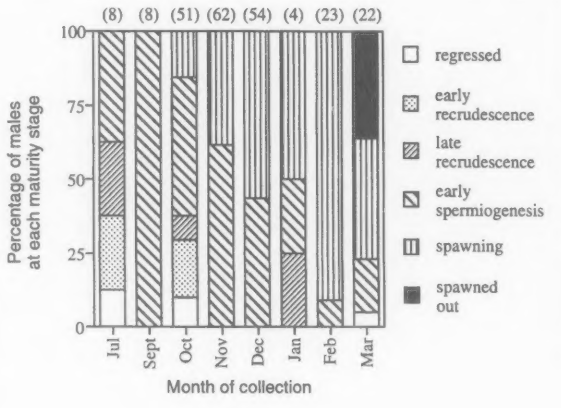


**Figure 3**

Percentage of male English sole at each stage of testicular development by collection month. Numbers in parentheses represent animals sampled each month and histologically analyzed for testicular stages.

## Discussion

Typical size-at-maturity for English sole is 260 and 210 mm TL for female and male sole, respectively (Garrison and Miller, 1982). Histological analyses of gonads collected in our study showed the smallest group of vitellogenic females to be 240–249 mm TL, but generally fish less than 260 mm TL were immature. Also, among females >260 mm, which were presumably old enough to undergo sexual maturation, approximately 24% were found in the regressed stage even during the peak of the reproductive season. This finding is consistent with findings by Johnson et al. (1988), in which the percentage of vitellogenic females at reference areas during peak recrudescence was about 80%. In contrast, males at much smaller length (i.e. 195 mm) were maturing, but no spawned out males were found at sizes <230 mm, suggesting that males <230 mm may not migrate to the spawning area. Although fish age was not determined in this study, length-at-age relationships determined for Puget Sound English sole (Myers et al., 1993) showed

that females at 260 mm were three years of age, and males at 195 mm were two years of age. Other studies have shown that females first mature at three years of age, whereas males mature at two years of age (Smith, 1936; Harry, 1959).

The pattern and timing of ovarian development observed in our study were similar to previous reports on English sole (Garrison and Miller, 1982; Kruse and Tyler, 1983; Johnson et al., 1991; Fargo and Tyler, 1994). Gonadal recrudescence in Puget Sound English sole began in early fall (September–October), vitellogenesis and spermiogenesis peaked in early winter (December–January), and spawning took place during late winter months (February and March). However, our findings suggest that small proportions of animals did enter vitellogenesis early; vitellogenic sole were found as early as July, whereas spawned out sole were found as early as October. In an earlier study that investigated oocyte development in female English sole (Johnson et al., 1991), the earliest spawning females were found in January. This discrepancy may be due to the fact that the number of fish collected in early fall in the first study (Johnson et al., 1991) was small compared with the present study; therefore, early spawning females may not have been observed. Nevertheless, changes in reproductive parameters (GSI, and plasma sex steroids) were similar to those described by Johnson et al. (1991) and to the reproductive endocrine cycles described for other species of female flatfish (e.g. Liu et al., 1991; Methven and Crim, 1991; Harmin et al., 1995). Generally, regressed fish had low values for the various reproductive parameters. The onset of vitellogenesis was accompanied by an increase in GSI and plasma sex steroid levels. After spawning, these levels declined to levels similar to those observed in regressed animals. Reproductive parameters at the two residential sites (Pilot Point and Tulalip Bay) were similar and supported the findings of Laroche and Richardson (1979) who observed no apparent latitudinal trend in the time of spawning in sole from the Oregon coast. Reproductive parameters in fish from University Point, however, were higher because a majority of sole at this site were undergoing final maturation or spawning (Johnson et al., 1991).

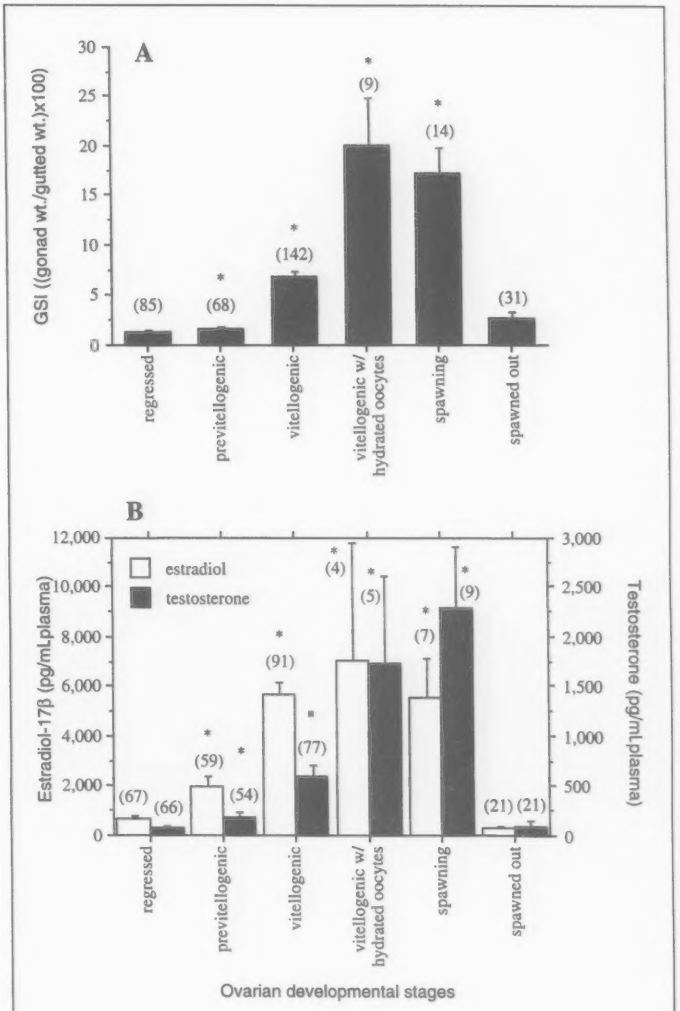


**Figure 4**

Mean ±SE of (**A**) gonadosomatic index (GSI), and (**B**) plasma steroid concentrations in female English sole at each stage of oocyte development. * denotes significantly higher levels than levels observed in regressed and spawned out fish (ANOVA, Fisher's PLSD, 95% CI). Numbers in parentheses represent animals at each ovarian developmental stage.

It is well established that E2 stimulates the liver to produce vitellogenin (Ng and Idler, 1983), and in this study, as in Johnson et al. (1991), vitellogenesis in female English sole coincided with a rise in plasma E2 concentrations. Plasma concentrations of T, a biosynthetic precursor to E2 (Kagawa et al., 1982), changed in parallel with the plasma E2 concentrations, although plasma T levels were lower than plasma E2 levels at all stages of gonadal development. Higher plasma T levels were observed in

vitellogenic sole compared with regressed sole, and highest plasma T concentrations were observed in spawning sole. Similar observations have been made in winter flounder *(Pleuronectes americanus)*, where concentrations of T have been correlated with oocyte stages characterized by germinal vesicle migration (Truscott et al., 1992b). In salmonids, estrogens and androgens such as T appear to play an important role in regulating production of pituitary GTH-II (Xiong et al., 1993, 1994), which stimulates the production of progestins, steroids involved in inducing final maturation (Swanson, 1991). This would be consistent with the high concentrations of T observed in spawning female English sole and winter flounder.

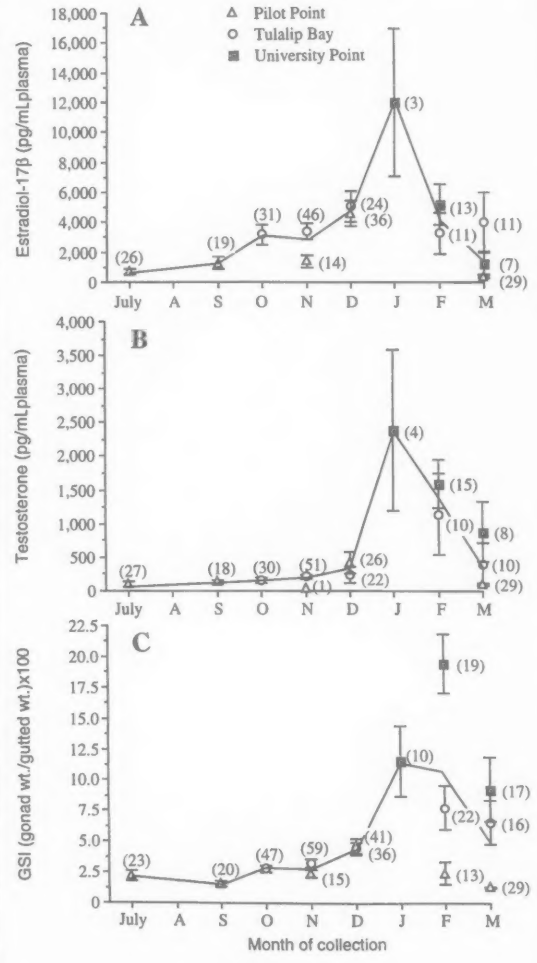


**Figure 5**

Mean ±SE of (**A**) gonadosomatic index (GSI), (**B**) plasma estradiol 17-β (E2) concentrations, and (**C**) plasma testosterone (T) concentrations in female sole from Tulalip Bay, Pilot Point, and University Point at each month of fish collection. Line represents mean levels at each month of fish collection. Numbers in parentheses represent animals sampled each month at each site.

In male teleosts, 11KT is known to be important in stimulating spermatogenesis, whereas T is known to be important in feedback effects on the pituitary (Borg, 1994). T is a biosynthetic precursor to 11KT (Ozon, 1972). As with E2 in female sole, the levels of T and 11KT covaried in male sole; 11KT levels were significantly higher than T levels at all stages of gonadal development. Similar relations between T and 11KT concentrations have been observed in other flatfish species, such as Pacific halibut *(Hippoglossus stenolepis)* (Liu et al., 1991) and winter flounder (Harmin et al., 1995). These results are consistent with the role of 11-oxygenated androgens as the dominant regulatory androgens in male teleosts, stimulating secondary sexual characters, reproductive behavior, and spermatogenesis (Borg, 1994).

In male winter flounder (Harmin et al., 1995) and plaice (Wingfield and Grimm, 1977), reproductive parameters (GSI, plasma 11KT and T concentrations) increased with the onset of seasonal testicular recrudescence, reached a peak in prespawning and spawning males, then decreased in spawned out males. However, in most male teleosts that have been studied, the levels of plasma 11KT and T peak during the prespawning period rather than during the spawning period (Borg, 1994). For example, the levels of plasma 11KT and T in Atlantic halibut *(Hippoglossus hippoglossus)* peaked briefly in sperm-producing fish, then declined during the spawning period (Methven and Crim, 1991). The reproductive parameters measured in male English sole were similar to the patterns observed in winter flounder and plaice, where steroid concentrations remained high during the peak spawning period.

Interestingly, the reproductive steroid levels observed in spawned out male English sole were significantly lower than levels observed in regressed males that were collected in summer and early fall. A pattern somewhat like this was also observed in male winter flounder and Atlantic halibut. In both male winter flounder (Harmin et al., 1995) and Atlantic halibut (Methven and Crim, 1991), the plasma steroid levels were low in spent males but began to increase about two months after the peak spawning period. Although this study did not measure reproductive parameters in the months immediately following the peak spawning period (i.e. April through June), similar changes

in plasma steroid levels may have occurred in male English sole and would account for the significant difference in plasma steroid levels between spent and regressed males.

A few male English sole sampled in July expressed milt even though reproductive steroid levels were low. Release of milt was also observed in winter flounder (Harmin et al., 1995) and Atlantic halibut (Methven et al., 1991) a few months earlier than vitellogenesis in females. Moreover, in winter flounder (Harmin et al., 1995), milt remained expressible a few months after the spawning season even though steroid levels were low during this time. Histological analyses of English sole testis showed that initiation of spermiogenesis does begin in July in a substantial proportion of sole, in spite of their low plasma steroid concentrations. This phenomenon has also been observed in other male fish, but the mechanism accounting for it is not entirely clear. Borg (1994) suggests that concentrations of T and 11KT in the testis itself may be quite high in early spermatogenesis, sufficient to stimulate spermiogenesis even when plasma concentrations of T and 11KT are low. The early production of mature sperm may be typical of most northern latitude, temperate zone teleosts in which fully developed spermatozoa are formed before winter and stored for discharge in spring (Lofts, 1987).

It is unclear which steroid induces final oocyte maturation in English sole. In all teleosts which have been studied, final maturation is triggered by a surge in plasma concentrations of a maturational gonadotropin (GTH-II). GTH-II stimulates gonadal production of progesterones and related compounds, the C21 steroids, which induce final oocyte maturation and production of sperm (Swanson, 1991). In most teleosts, 17α, 20β-P is believed to be a potent MIS (Scott and Canario, 1987, 1992; Inbaraj et al., 1995). However, in flatfish species that have been studied, low or nondetectable levels of 17α, 20β-P were found (Howell and Scott, 1989; Truscott et al., 1992b; Mugnier et al., 1995). Similarly, 17α, 20β-P was not detected in spawning English sole, and in some flatfish species, other steroids have been suggested as the MIS. For example, in winter flounder 17β-hydroxy-5β-androstan-3-one and T were correlated with oocyte stages characterized by germinal vesicle migration (Truscott et al., 1992b), whereas in turbot *(Scophthalmus maximus L.)* 17α, 21β,21-trihydroxy-4-pregnene-3-one-20 has been shown to induce final maturation (Mugnier et al., 1995). Recent studies also suggest that in plaice *(Pleuronectes platessa)* and Dover sole *(Solea solea)* 17α, 20β-P actually does induce final maturation but is metabolized before it can be detected in

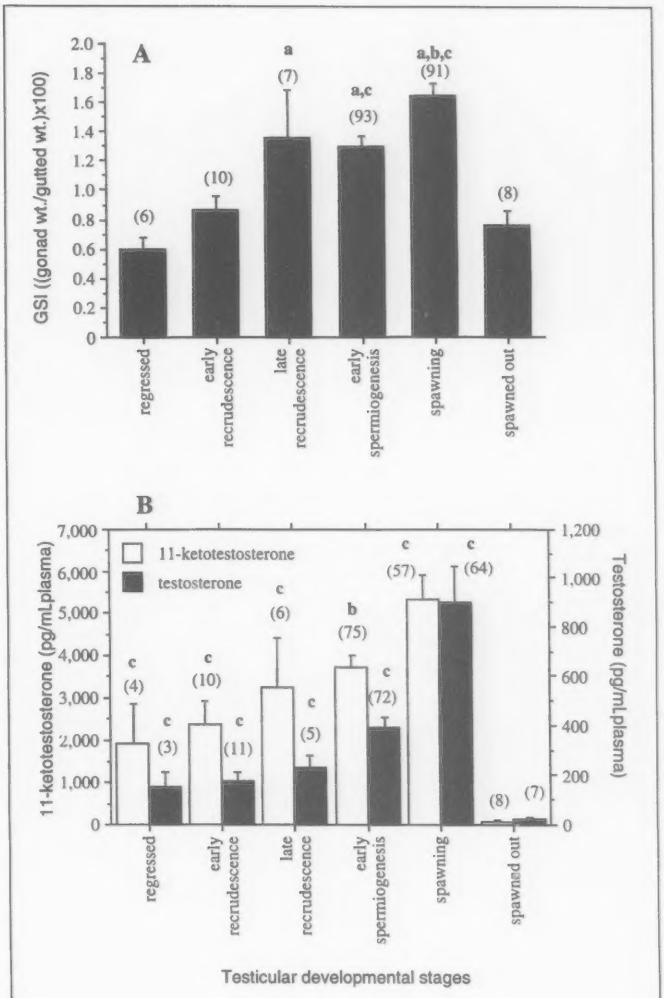


**Figure 6**

Mean ±SE of (**A**) gonadosomatic index (GSI) and (**B**) plasma steroid concentrations in male English sole at each stage of testicular development. "a" denotes significantly higher levels than levels observed in regressed fish; "b" denotes significantly higher levels than levels observed in early recrudescence fish; and "c" denotes significantly higher levels than levels observed in spawned out fish (ANOVA, Fisher's PLSD, 95% CI). Numbers in parentheses represent animals at each testicular developmental stage.

the bloodstream with conventional RIAs (Scott and Canario, 1992; Inbaraj et al., 1995). Clearly, additional research is needed in this aspect of English sole reproductive biology.

In summary, the reproductive endocrine cycle in female and male English sole from two residential sites and a spawning site in Puget Sound, WA, was characterized by measuring reproductive steroid concentrations and correlating them with collection time and histological assessment of gonadal stage. This study provides the first description of the reproductive endocrine cycle in male English sole and can be used as a baseline tool in evaluating the effects of contaminants on reproductive endocrine function in male English sole, or in individuals used in mariculture.

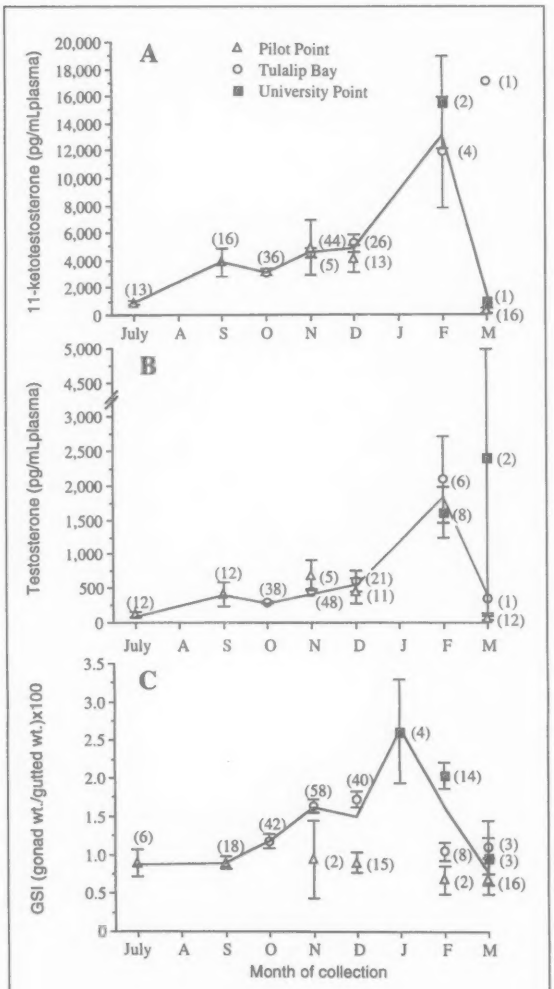


**Figure 7**

Mean ± SE of (**A**) gonadosomatic index (GSI), (**B**) plasma 11-ketotestosterone (11KT) concentrations, and (**C**) plasma testosterone (T) concentrations in male sole from Tulalip Bay, Pilot Point, and University Point at each month of fish collection. Line represents mean levels at each month of fish collection. Numbers in parentheses represent animals sampled each month at each site.

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## Literature cited

**Billard, R.**
**1992.** Reproduction in rainbow trout: sex differentiation, dynamics of gametogenesis, biology, and preservation of gametes. Aquaculture 100:263–297.

**Borg, G.**
**1994.** Androgens in teleost fishes. Comp. Biochem. Physiol. 109:219–245.

**Casillas, E. D., L. Misitano, Johnson, L. Rhodes, T. Collier, J. Stein, B. McCain, and U. Varanasi.**
**1991.** Inducibility of spawning and reproductive success of female English sole *(Parophrys vetulus)* from urban and nonurban areas of Puget Sound, Washington. Mar. Environ. Res. 31:99–122.

**Collier, T. K., L. L. Johnson, C. M. Stehr, M. S. Myers, and J. E. Stein.**
**In press.** A comprehensive assessment of the impacts of contaminants on fish from an urban waterway. Mar. Environ. Res.

**Collier, T. K., J. E. Stein, H. R. Sanborn, T. Hom, M. S. Myers, and U. Varanasi.**
**1992.** Field studies of reproductive success and bioindicators of maternal contaminant exposure in English sole (*Parophrys vetulus*). Sci. Tot. Environ. 116:169–185.

**Dowdy, S., and S. Weardon.**
**1991.** Statistics for research. John Wiley and Sons, New York, NY, 629 p.

**Fargo, J., and A.V. Tyler.**
**1994.** Oocyte maturation in Hecate Strait English sole *(Pleuronectes vetulus)*. Fish. Bull. 90(1):189–197.

**Garrison, K. J., and B. S. Miller.**
**1982.** Review of the early life history of Puget Sound fishes. Univ. Washington, School of Fisheries Research Institute, Seattle, WA, 729 p.

**Gray, P.**
**1954.** The microtomists' formulatory and guide. Blakiston, NY, 808 p.

**Harmin, S. A., L. W. Crim, and M. D. Wiegand.**
**1995.** Plasma sex steroid profiles and the seasonal reproductive cycle in male and female winter flounder, *Pleuronectes americanus* (Walbum). Mar. Biol. 121: 601–610.

**Harry, G. Y.**
**1959.** Time of spawning, length at maturity, and fecundity of English, petrale, and Dover sole (*Parophrys vetulus, Eopsetta jordani,* and *Microstomus pacificus,* respectively). Fish. Comm. Oreg. Res. Briefs 7:5–13.

**Howell, R., and A. P. Scott.**
**1989.** Ovulation cycles and post-ovulatory deterioration of eggs of the turbot (*Scophthalmus maximus* L.). Rapp. Reun. Cons. Int. Explor. Mer 191:21–26.

**Idler, D. R., Y. P. So, G. L. Fletcher, and J. F. Payne.**
**1995.** Depression of blood levels of reproductive steroids and glucuronides in male winter flounder *(Pleuronectes americanus)* exposed to small quantities of Hibernia crude, used crankcase oil, oily drilling mud, and harbour sediments in the 4 months prior to spawning in late May–June. *In* F. W. Goetz and P. Thomas (eds.), Proc. 5th int. symp. reprod. physiol. fish, p.187. Univ. Texas, Austin, TX.

**Inbaraj, R. M., A. P. Scott, and E. L. M. Vermeirssen.**
**1995.** Monitoring final oocyte maturation in female plaice *(Pleuronectes platessa)* using RIAs which detect metabolites of 17α, 20β-dihydroxy-4-pregnen-3-one. *In* F. W. Goetz and P. Thomas (eds.), Proc. 5th int. symp. repro. physiol. fish, p. 311. Univ. Texas, Austin, TX.

**Jobling, S., D. Sheahan, J. A. Osborne, P. Matthiessen, and J. P. Sumpter.**
**1996.** Inhibition of testicular growth in rainbow trout *(Oncorhynchus mykiss)* exposed to estrogenic alkylphenolic chemicals. Environ. Toxicol. 15(2):194–202.

**Johnson, L. L., and E. Casillas.**
**1991.** The use of plasma parameters to predict ovarian maturation stage in English sole *Parophrys vetulus* Girard. J. Exp. Mar. Biol. Ecol. 151:257–270.

**Johnson, L., E. Casillas, T. Collier, B. McCain, and U. Varanasi.**
**1988.** Contaminant effects on ovarian maturation in English sole (*Parophrys vetulus)* from Puget Sound, Washington. Can. J. Fish. Aquat. Sci. 45:2133–2146.

**Johnson, L. L., E. Casillas, T. K. Collier, J. E. Stein, and U. Varanasi.**
**1993.** Contaminant effects on reproductive success in selected benthic fish species. Mar. Environ. Res. 35:165–170.

**Johnson, L. L., E. Casillas, M. S. Myers, L. D. Rhodes, and O. P. Olson.**
**1991.** Patterns of oocyte development and related changes in plasma 17β-estradiol, vitellogenin, and plasma chemistry in English sole *Parophrys vetulus* Girard. J. Exp. Mar. Biol. Ecol. 152:161–185.

**Kagawa, H., G. Young, and Y. Nagahama.**
**1982.** Estradiol 17-β production in isolated amago salmon *(Onchorhynchus rhodurus)* ovarian follicles and its stimulation by gonadotropins. Gen. Comp. Endocrinol. 47:361–365.

**Ketchen, K. S.**
**1947.** Studies on lemon sole development and egg production. Fish. Res. Board Can. Prog. Rep. Prog. Rep. Pac. Coast Sta. 73:68–70.

**Kruse, G. H., and A. V. Tyler.**
**1983.** Simulation of temperature and upwelling effects on the English sole *(Parophrys vetulus)* spawning season. Can. J. Fish. Aquat. Sci. 40:230–237.

**Laroche, J. L., and S. L. Richardson.**
**1979.** Winter-spring abundance of larval English sole, *Parophrys vetulus*, between the Columbia River, and Cape Blanco, Oregon, during 1972–1975 with notes on occurrences of three other pleuronectids. Estuarine Coastal Mar. Sci. 8(5):455–476.

**Liu, H., R. Stickney, and W. Dickoff.**
**1991.** Changes in plasma concentrations in adult Pacific halibut, *Hippoglossus stenolepis.* J. World Aquacult. Soc. 22(1):30–35.

**Lofts, B.**
**1987.** Testicular function. *In* D. O. Norris and R. E. Jones (eds.), Hormones and reproduction in fishes, amphibians and reptiles, p. 283–325. Plenum, New York, NY.

**Luna, L. G. (ed.).**
**1968.** Manual of histologic staining methods of the Armed Forces Institute of Pathology, third edition. McGraw-Hill, New York, NY, 206 p.

**Methven, D. A., and L. W. Crim.**
**1991.** Seasonal changes in spermatocrit, plasma sex steroids, and motility of sperm from Atlantic halibut *(Hippoglossus hippoglossus).* *In* A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe (eds.), Proc. 4th int. symp. reprod. physiol. fish, p. 170. Univ. East Anglia, Sheffield, UK.

**Mugnier, C., J. L. Gaignon, E. Lebegue, C. Fauvel, and A. Fostier.**
**1995.** Maturation inducing steroid in turbot (*Scophthalmus maximus* L). *In* F. W. Goetz and P. Thomas (eds.), Proc. 5th int. symp. reprod. physiol. fish, p. 323. Univ. Texas, Austin, TX.

**Myers, M. S., C. M. Stehr, O. P. Olson, L. L. Johnson, B. B. McCain, S-L. Chan, and U. Varanasi.**
**1993.** National status and trends program, national benthic surveillance project: Pacific Coast. Fish histopathology and relationships between toxicopathic lesions and exposures to chemical contaminants for cycles I to V (1984–88). U.S. Dep. Commerce, NOAA Tech. Memo. NMFS-NWFSC-6, 160 p.
**1994.** Relationship between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole *(Pleuronectes vetulus)*, starry flounder *(Platichthys stellatus)*, and white croaker *(Genyonemus lineatus)* from selected marine sites on the Pacific Coast, USA. Environ. Health Perspect. 102(2):200–215.

**Ng, T. G., and D. R. Idler.**
**1983.** Yolk formation and differentiation in teleost fishes. *In* W. S. Hoar, D. J. Randall, and E. M. Donaldson (eds.), Fish physiology, vol. IX, Reproduction pt. A, p. 373–403. Academic Press, New York, NY.

**Nimrod, A. C., and W. H. Benson.**
**1996.** Environmental estrogenic effects of alkylphenol ethoxylates. Crit. Rev. Toxicol. 26(3):335–364.

**Ozon, T.**
**1972.** Androgens in fishes, amphibians, reptiles and birds. *In* D. R. Idler (ed.), Steroids in nonmammalian vertebrates, p. 329–389. Academic Press, New York, NY.

**Scott, A. P., and A.V. M. Canario.**
**1987.** Status of oocyte maturation-inducing steroids in teleosts. *In* D. R. Idler, L. W. Crim, and J. M. Walsh (eds.), Proc. 3th int. symp. repro. physiol. fish, p. 224–234. Marine Sciences Research Laboratory, St. John's, Newfoundland, Canada.
**1992.** 17α, 20β-dihydroxy-4-pregnen-3-one 20-sulfate: a major new metabolite of the teleost oocyte maturation-inducing steroid. Gen. Comp. Endocrinol. 85:91–100.

**Scott, A. P., E. L. Sheldrick, and A. P. Flint.**
**1982.** Measurement of 17α, 20β-dihydroxy-4-pregnen-3-one in plasma of trout *(Salmo gairdneri* Richardson): seasonal changes and response to salmon pituitary extract. Gen. Comp. Endocrinol. 46:444–451.

**Smith, R.T.**
**1936.** Report on the Puget Sound otter trawl investigations. State of Washington. Dep. Fisheries. Biological Report 36B, 42 p.

**Sower, S. A., and C. B. Schreck.**
**1982.** Steroid and thyroid hormones during sexual maturation of coho salmon *(Oncorhynchus kisutch)* in seawater or freshwater. Gen. Comp. Endocrinol. 47:42–53.

**Swanson, P.**
**1991.** Salmon gonadotropins: reconciling old and new ideas. *In* A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe (eds.), Proc. 4th int. symp. repro. physiol. fish, p. 2–7. Univ. East Anglia, Sheffield, UK.

**Truscott, B., D. R. Idler, and G. L. Fletcher.**
**1992a.** Alteration of reproductive steroids of male winter flounder *(Pleuronectes americanus)* chronically exposed to low levels of crude oil in sediments. Can. J. Fish. Aquat. Sci. 49:2190–2195.

**Truscott, B., Y. So, J. Nagler, and D. Idler.**
**1992b.** Steroids involved with final oocyte maturation in the winter flounder. J. Steroid Biochem. Mol. Biol. 42:351–356.

**Wingfield, J. C., and A. S. Grimm.**
**1977.** Seasonal changes in plasma cortisol, testosterone and oestradiol-17β in the plaice, *Pleuronectes platessa L.* Gen. Comp. Endocrinol. 31:1–11.

**Xiong, F., R. Chin, Z-Y. Gong, K. Suzuki, R. Kitching, S. Majumdar-Sonnydal, H. Elsholtz, and C.L. Hew.**
**1993.** Control of salmon pituitary hormone gene expression. Fish Physiol. Biochem. 11:63–70.

**Xiong, F., D. Liu, Y. Le Drean, H. Elsholtz, and C. L. Hew.**
**1994.** Differential recruitment of steroid hormone response elements may dictate the expression of the pituitary gonadotropin IIβ subunit gene during salmon maturation. Mol. Endocrinol. 8:782–793.

# Parasitism of the golden king crab, *Lithodes aequispinus*, by two species of snailfish, genus *Careproctus*

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**Abstract.**—Along the continental slope of the eastern Bering Sea, two species of *Careproctus* snailfishes deposit eggs within the branchial chambers of the commercially important golden king crab (*Lithodes aequispinus*). The larger of the two species is the pink snailfish (*C. furcellus*); the smaller species is an undescribed species referred to as the red snailfish. According to 1982 trawl survey data, incidence of snailfish eggs and larvae within crab branchial chambers increases with carapace length (CL) and is greater for male than female crabs. A logistic model fitted to the incidence data predicts that a 140-mm-CL male will have an incidence of 0.52, approximately 1.9 times greater than the incidence for a 100-mm male. Incidence for a 100-mm-CL male is approximately 1.9 times greater than that for a female of equal size. On the basis of developmental stages of embryos carried by female golden king crab and the developmental stages of snailfish embryos within a female's branchial chambers, snailfish appear to deposit eggs preferentially in crabs that are early in their molt cycle. The presence of the egg masses results in gill compression, localized necrosis of gill tissue and, in extreme cases, total loss of gill tissue on one side of the body. For crabs of commercial size, the presence of eggs and larvae increases mortality within the holds of fishing vessels by 35%. The current incidence of eggs and larvae in commercial sized males, however, is so low that the effect on the commercial fishery is considered to be small.

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Fish. Bull. 96:871–884 (1998).

Liparid snailfish in the genus *Careproctus* extrude eggs through an anteriorly positioned ovipositor into the branchial chambers of large lithodid crabs. This relationship was first described, independently, by Rass (1950) and Vinogradov (1950) for *C. sinensis* and *Paralithodes camtschatica* in the northwest Pacific. Subsequent studies, however, have shown that additional species both within *Paralithodes* as well as within three other lithodid genera (*Lithodes, Lopholithodes* and *Paralomis*), also serve as hosts to various *Careproctus* species and that the association appears to be widespread according to reports from the North and South Pacific and South Atlantic (Hunter, 1969; Parrish, 1972; Peden and Corbett, 1973; Anderson and Cailliet, 1974; Balbontin et al., 1979; Melville-Smith and Louw, 1987; and Love and Shirley, 1993).

The advantages that snailfish gain by placing their eggs within an enclosed, constantly aerated space have been recognized since the association between *Careproctus* and lithodid crabs was discovered (Rass, 1950). The disadvantages to the crab, however, remain equivocal, with reports of no obvious damage (Hunter, 1969; Parrish, 1972), minor gill compression by the egg mass (Anderson and Cailliet, 1974; Melville-Smith and Louw 1987), and gill bleeding (Love and Shirley, 1993). Knowledge of the disadvantages is important because most affected lithodid species support commercial fisheries.

In this paper, we report on the deposition of egg masses by two species of *Careproctus* into the branchial chambers of the golden king crab (*Lithodes aequispinus*), a large, commercially harvested lithodid occurring along the continental slope of the North Pacific (Somerton and Otto, 1986). The identity of the two *Careproctus* species is uncertain because the taxonomy of the family Liparididae is problematic and incomplete (Allen and Smith, 1988). The larger species (Fig. 1), henceforth called the pink snailfish, is certainly within the *C. melanurus* group of morphologically similar species (Allen and Smith, 1988) and is likely to be *C. furcellus* which has been observed throughout the Bering Sea and Aleutian Islands (Kido, 1988). The smaller species (Fig. 1), henceforth called the red snailfish, is tentatively identified as a member of the *C. mederi* group described by Kido (1988), who re-

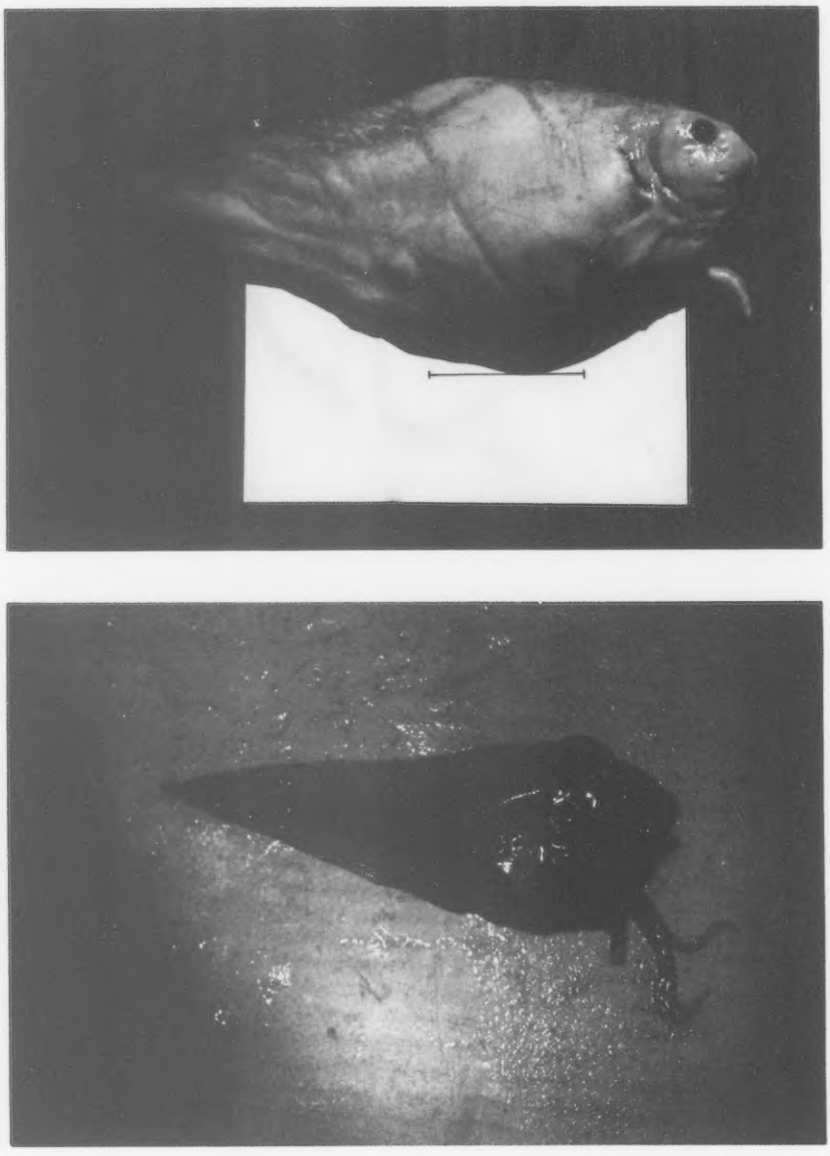

**Figure 1**

(Upper) pink snailfish (*Careproctus furcellus*); (lower) red snailfish (*C.* species). Both specimens are ripe females with their ovipositors extended. Note that the red snailfish has extremely long lobes on the pectoral fins that, in the picture, have been positioned in front of the body for clarity.

ferred to it as *Careproctus* species B.[1] Here we first examine the characteristics of this association, then consider how *Careproctus* snailfish choose their host crabs and what the consequences may be to the crabs.

[1] Stein, D. 1996. National Marine Fisheries Service, 1315 East-West Hwy., Silver Springs, MD, 20910. Personal commun.

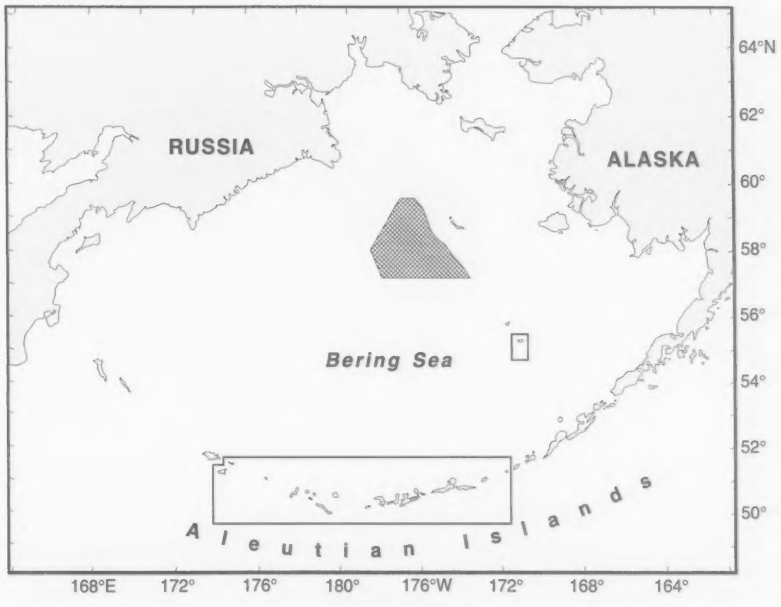


**Figure 2**

Collecting sites for the 1982 trawl survey (shaded area) and the 1996 commercial catch sampling areas in the Bering Sea and Aleutian Islands (unshaded areas).

## Materials and methods

Golden king crab were sampled 11 July–25 August 1982 aboard the FV *Rujin Maru no. 8* while it was conducting a bottom trawl survey of the benthic resources along the continental slope of the eastern Bering Sea (Fig. 2). All golden king crab were first sorted to sex, then measured for carapace length (CL) from the base of the eye to the posterior midline of the carapace. Females were evaluated for maturity, on the basis of relative width of their abdomens, and if mature, their pleopods were examined to determine whether they were carrying uneyed embryos, eyed embryos, or remnants of recently hatched embryos (egg cases and funiculi), or whether they were bare.

During this sampling, the senior author discovered that many of the crabs contained *Careproctus*-like egg masses or larvae within their branchial chambers. Further inspection of the egg masses and larvae revealed that there appeared to be two distinct types, one with egg diameters or larvae lengths considerably larger than the other. Several species of *Careproctus* were also captured in the trawls but two species, pink and red snailfish, predominated. Gentle pressure applied to the abdomens of some individuals of both species resulted in eggs being extruded through the ovipositor (Fig. 1) or milt being extruded out the genital papilla (Stein, 1980). The eggs of pink snailfish were visibly larger than those of red snailfish and the egg sizes of both species clearly matched the egg sizes found within the crabs. Consequently, starting on 27 July, 48 consecutive trawl hauls were sampled as follows for *Careproctus* spp. and their eggs and larvae.

All golden king crabs were examined for the presence of *Careproctus* egg masses or larvae clusters by removing their carapaces. Each mass was evaluated to type (uneyed embryos, eyed embryos, or larvae) and species (large eggs and larvae were assigned to pink snailfish; small eggs and larvae were assigned to red snailfish), and for location (i.e. which side of the crab it was obtained). Snailfish were first sorted to species, then externally sexed, if possible, by the presence of either an ovipositor or a genital papilla. Snailfish were then gently squeezed about the abdomen and classified as ripe if either eggs or milt were extruded. Thus, the sex could be either known or unknown and, if known, the individual could be either ripe or unripe.

The 1996 incidence of snailfish eggs and larvae within golden king crab were assessed by Alaska Department of Fish and Game at-sea observers and port samplers during the golden king crab fishing season in the Aleutian Islands and eastern Bering Sea. Female and small (<135 mm-CL) male golden

king crab, which are not retained by the fishery, were sampled aboard commercial vessels by occasionally collecting all individuals from one randomly chosen trap. Sampled crabs were first measured for carapace length, then examined for the presence of snailfish eggs and larvae, without regard to species, by removing their carapaces. Legal-size male crab were sampled from the commercial catches when they were landed at the processing plant. Catches were first separated into groups of live and dead crabs, then counted and subsampled by examining up to 100 individuals from each group for the presence of snailfish eggs. Port samples were collected for crabs caught either in the Bering Sea (three landings) or the Aleutian Islands (six landings; Fig. 2), but at-sea samples were collected only in the Aleutian Islands.

Thirteen golden king crabs found, during commercial sampling, to contain snailfish eggs or larvae were frozen whole, with the eggs or larvae left in place for later processing. Eighteen of 23 egg and larvae samples were nonhatched egg masses that were placed in water overnight to fully rehydrate, then weighed to the nearest 1 mg. Displacement volume of the egg masses (mL) was measured in a graduated cylinder. Egg masses were then teased apart and the total number of eggs counted. Diameters of approximately 200 eggs randomly chosen from each egg mass were measured to the nearest 0.03 mm with an optical micrometer. Volumes of the gills on both the affected and unaffected side of the crab were measured by displacement after they were excised with a scalpel at their bases. Volume of the empty branchial chamber on one side of the crab was then measured by securing the carapace in its proper place on the crab and then injecting Polycel insulating foam into the branchial cavity through a small hole. After any excess was removed, the volumes of the foam casts were then measured by displacement to the nearest milliliter. The 23 egg and larvae samples were then subjected to restriction fragment length polymorphism (RFLP) analysis (Dowling et al., 1996) by using six restriction enzymes to determine species identity.[2] Species identification patterns were established by using the RFLP analysis on six *Careproctus rastrinus*, eight *C. furcellus,* and one *C. cypselurus* collected in crab pots during commercial sampling.

The incidence (e.g. probability of occurrence) of snailfish eggs and larvae as a function of several predictor variables was described with a generalized linear model, with a logit link function and a binomial variance function (Venables and Ripley, 1994). For each crab, model inputs included a binary response variable (presence or absence of eggs or larvae). For the 1982 survey data, predictors included two continuous variables (carapace length and depth) and a discrete variable (sex). For the 1996 port sampling data, predictors included two discrete variables (area and whether the crab was alive or dead). Model selection was accomplished iteratively by first testing the significance of each interaction term with a likelihood ratio test, then by discarding the least significant term. This process was then repeated on each main effect and any associated interaction terms.

Differences in the depth distributions of male and female crab and of spawning and nonspawning snailfish were tested by first calculating a Cramer-von Mises statistic from catch-per-tow and depth data, then by determining the significance of the statistic with randomization (Syrjala, 1996).

Association between the developmental stage of eggs carried by a female golden king crab and the developmental stage of snailfish eggs within a crab's branchial chambers was tested with a Spearman's rank order correlation coefficient. Because king crabs extrude eggs onto their pleopods soon after molting (Powell and Nickerson, 1965; Sloan, 1985), the stage of embryonic development is a crude measure of the relative time since molting. Positive correlation between the developmental stages of crab and snailfish embryos would indicate that snailfish preferentially choose newly molted crabs as hosts. To simplify interpretation, 3 of 26 females were not used because they contained more than a single egg mass or larvae cluster. Embryonic developmental stages for snailfish (uneyed, eyed, and larval) and crabs (uneyed, eyed, and hatched) were considered as ordered variables and were assigned numeric codes (i.e. 1, 2, 3). Mature female crabs without any obvious egg remnants attached to the pleopods were grouped with females carrying hatched eggs because egg remnants may have been missed in the macroscopic examination performed at-sea.

The mortality rate of male crabs in the holding tanks of commercial vessels was estimated separately for crabs with and without snailfish eggs and larvae on the basis of 1996 port sampling data for commercial crab landings. For each landing, the total number of live crabs that were infested was estimated by multiplying the number of live crabs landed by the infested proportion in the catch subsample. The total number of infested dead crabs was estimated similarly. The mortality rate of infested and uninfested crabs was then estimated as the number dying divided by the total in each category. Average mortality rate was then estimated as the mean over all landings.

[2] Lopez, A. 1996. Marine Molecular Biological Laboratory, University of Washington, Seattle, WA. Personal commun.

## Results

### Depth distributions of golden king crabs and snailfish

The 48 hauls sampled on the 1982 survey were randomly distributed between 192 and 900 m. Over this range, golden king crab catch-per-tow (Fig. 3) and carapace length (Fig. 4) declined with increasing depth. Male and female crab occurred at significantly different depths (randomization test, $P$=0.005), with the median depth of males (203 m) being less than that of females (336 m).

Pink snailfish abundance declined with depth; most of the population occurred at depths <450 m (Fig. 5). Conversely, red snailfish abundance increased with depth; most of the population occurred at depths >450 m. (Fig. 5). Red snailfish was approximately six times more abundant, averaged over the entire survey area, than pink snailfish (mean catch per haul: red snailfish=4.57, pink snailfish=0.75, total number of both species=226). As a result, red snailfish were predominate at depths as shallow as 350 m. The proportion of the individuals that were ripe (both sexes combined) did not differ significantly between species (chi-square test, df=1, $P$=0.341). Ripe pink snailfish (both sexes combined) were found at significantly shallower depths than unripe individuals (randomization test, $P$<0.001), but ripe and unripe red snailfish did not differ in depth ($P$=0.255).

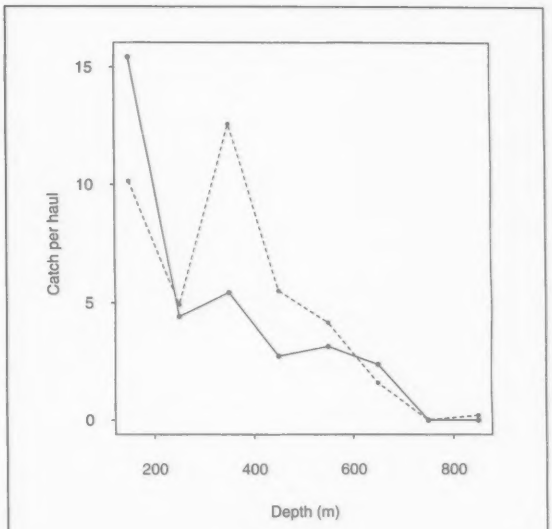


**Figure 3**

Catch per haul by 100-m depth intervals for male (solid) and female (dotted) golden king crab.

### *Careproctus* eggs and larvae

Snailfish eggs are self adhesive and form compact masses that are often shaped like casts of the interior of the branchial chambers (Fig. 6). After hatching, larvae remain grouped together within the crab, thus allowing multiple batches of larvae to be easily distinguished. Of the 515 golden king crab examined on the 1982 survey, 97 contained 128 egg masses or larvae clusters. Twenty-three of the crabs had more than one egg mass or larval cluster (16 had 2, 6 had 3, and 1 had 4 egg masses or larvae clusters). The abundance of multiple occurrences relative to single occurrences did not differ between snailfish species (chi-square test, df=1, $P$= 0.111), indicating that the two species were equally likely to deposit eggs in previously infested crabs. Combined over both species, the number of egg masses found within infested crabs increased with carapace length (linear regression, $n$=94, $P$=0.049), indicating that large crabs tended to have more multiple occurrences. In three cases of multiple occurrences, eggs or larvae of both snailfish species were present. The relative abun-

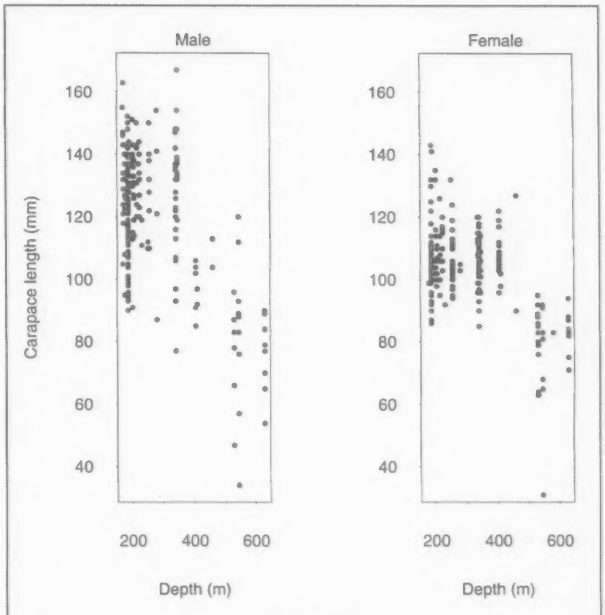


**Figure 4**

Carapace length of all crabs captured as a function of depth for male (left) and female (right) golden king crab.

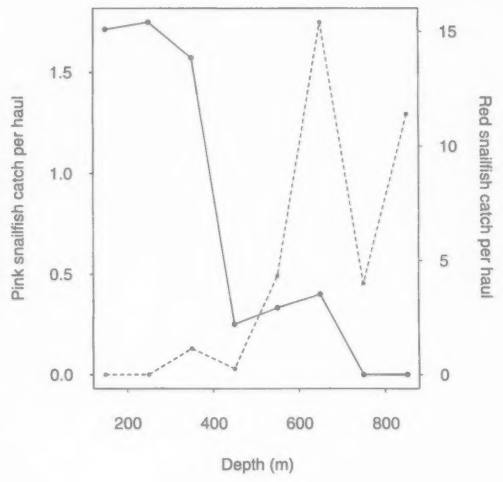


**Figure 5**

Catch per haul by 100-m depth intervals for pink snailfish (solid) and red snailfish (dotted).

dance of the three developmental stages (uneyed eggs, eyed eggs, and larvae) did not differ between snailfish species (chi square test, $P$=0.163), indicating that the timing of the egg deposition events and subsequent embryonic and larval developmental rates were about the same for both species. Summed over both species, the relative abundance of uneyed and eyed embryos was about equal, whereas the abundance of larvae was less than 20% that of either embryonic stage (Table 1).

Mean egg diameter was 4.83 mm (SD=0.29, eggs=4207, egg masses=18) and mean egg number was 688 (SD=84.0, egg masses=15). By comparison, the mean diameter of eggs taken from the ovarian lumen of a single pink snailfish was 4.9 mm (SD=0.124, $n$=208) and the total number of eggs was 790 with a displacement volume of 48.2 mL. Based on this specimen, mean egg volume was 0.061 mL (SD=0.017) and mean egg mass was 0.064 gm (SD=0.020).

Volume of single egg masses increased asymptotically with branchial volume (Fig. 7), indicating that up to some crab sizes, egg mass volume is limited by the available space in the branchial chambers. Up to 65% of the available volume within the branchial chambers (i.e. branchial volume – gill volume) was filled by a single egg mass.

**Table 1**

Incidence of *Careproctus* egg masses and larval clusters by species and stage of development.

| | Uneyed embryos | Eyed embryos | Larvae | Total |
|---|---|---|---|---|
| Red snailfish | 36 | 45 | 6 | 87 |
| Pink snailfish | 24 | 14 | 3 | 41 |
| Total | 60 | 59 | 9 | 128 |

## Incidence of snailfish eggs and larvae in golden king crab

Before developing a statistical model describing the 1982 incidence of snailfish eggs and larvae as a function of crab sex, carapace length, and sampling depth, we performed statistical tests to determine whether the two snailfish species could be combined in the analysis. Incidence of pink and red snailfish eggs did not differ by sex of host (chi-square test, df=1, $P$=0.28), nor by size of host ($t$-test, df=128, $P$=0.58), but incidence did differ by depth. Pink snailfish occurrences declined with increasing depth until none were encountered at depths >400 m (Fig. 8). In contrast to this, red snailfish masses increased in abundance with depth until reaching a peak at between 300–400 m, then declined until none were encountered at depths >600 m. Despite the difference in depth distribution for each species, the species were combined to simplify analysis. Because the combined incidence of eggs and larvae increased with depth to a peak at 250 m, then subsequently declined (Fig. 8), the depth effect in the model included a quadratic term. The best fit of a logistic model to the combined incidence data indicated that sex ($P$<0.001), length ($P$=0.003), and depth ($P$<0.001) were all highly significant (Table 2). The fitted model predicts 1) incidence increases with size (Fig. 9), 2) incidence is greater for males than females (Fig. 9), and 3) incidence is greater at middepth (i.e. significant quadratic depth effect, Table 2).

The best fit of a logistic model to the 1996 incidence in commercial males indicated that area ($P$<0.001) and whether the crabs were landed dead or alive ($P$=0.046) were both significant (Table 2). Incidence in the Bering Sea was over three times greater than in the Aleutian Islands. Incidence in dead crabs was 1.9 times greater than in live crabs in the Bering Sea and 1.6 times greater in the Aleutian Islands (Table 3). Incidences in live commercial males from the Aleutian Islands were 2.5 times greater than in sublegal males and 12.5 times greater than in females (Table 3). Incidence in 1996 of live commercial males in the Bering Sea was less than 20% of the incidence in 1982 commercial size males

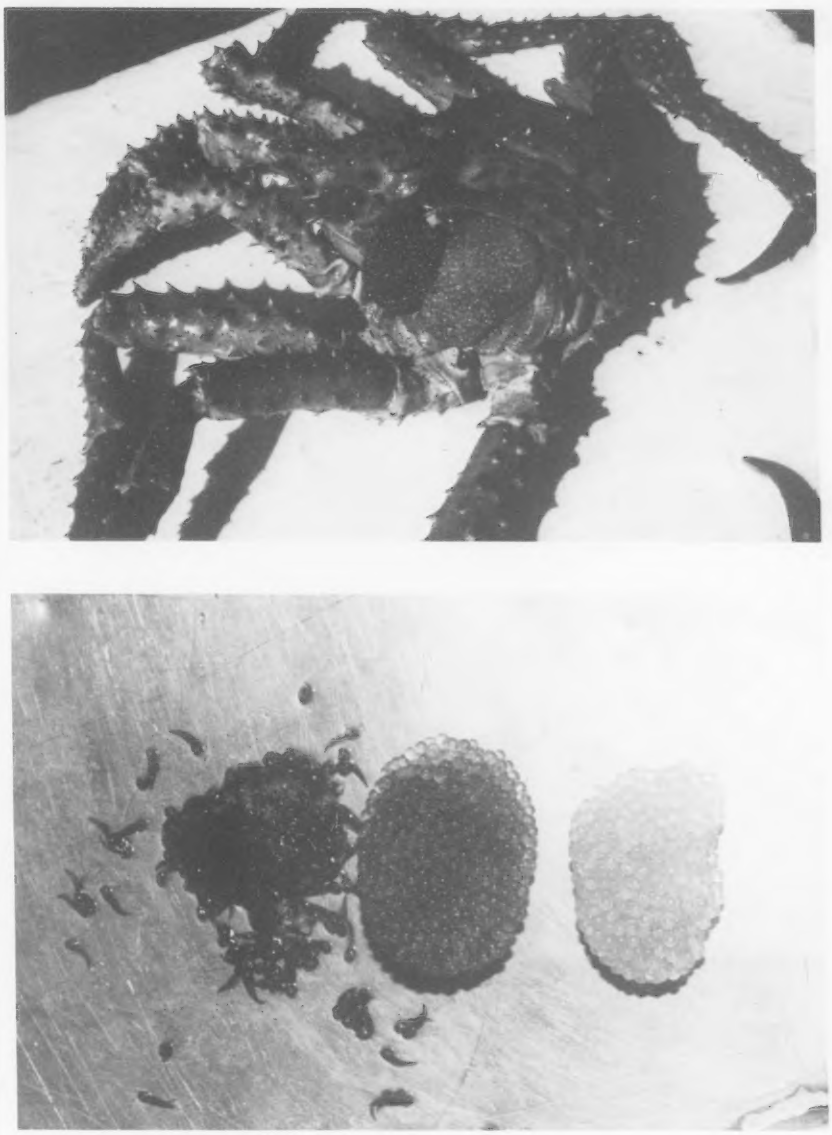

**Figure 6**

(Upper) A male golden king crab with part of the carapace cut away to show two *Careproctus* egg masses in different stages of development. (Lower) An early uneyed egg mass (right), a late uneyed egg mass (center), and a larvae cluster (left) taken from a single golden king crab.

(CL>135 mm; Table 3). The probability of a male dying in the holding tank of a commercial vessel was 0.035 if the crab contained snailfish eggs and larvae and 0.025 if the branchial chambers were empty, indicating that snailfish infestion increases the holding mortality of crabs.

## Association between crab and snailfish embryonic development stages

Mature female golden king crab carried snailfish embryos in all three stages of development (uneyed, eyed, and larval) only when they themselves were

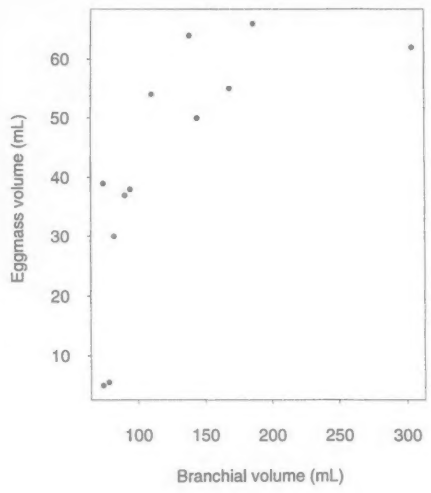


**Figure 7**

Volume of single egg masses as a function of the volume of the branchial chambers of the host crab.

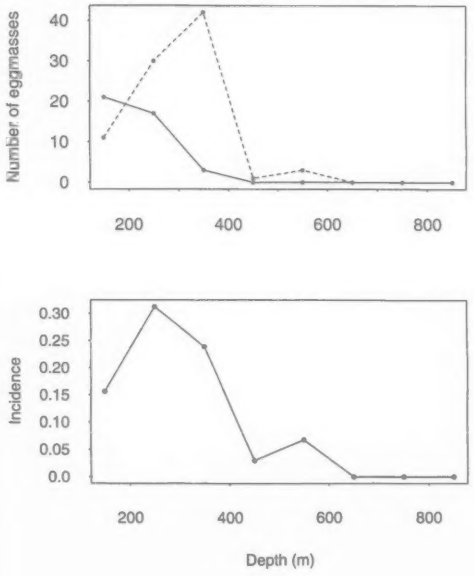


**Figure 8**

Number of egg masses by 100-m depth intervals for pink snailfish (solid line) and red snailfish (dotted line, upper). Incidence, or proportion of all crabs having snailfish eggs or larvae of either species, by 100-m depth interval (lower).

**Table 2**

Summary statistics of the fitted logistic models.

**Model 1 (1982 survey data)**

logit( incidence) = intercept + sex + length + depth + depth$^2$ + sex*depth + sex × depth$^2$

| Coefficient | Value |
|---|---|
| Intercept | –8.6724639 |
| Sex | –2.2823274 |
| Length | 0.0261672 |
| Depth | 0.0310400 |
| Depth$^2$ | –0.0000432 |
| Sex × depth | 0.0187658 |
| Sex × depth$^2$ | –0.0000512 |

Significance tests of main effects

| Effect | Likelihood ratio | df | Probability |
|---|---|---|---|
| Length | 8.87 | 1 | 0.003 |
| Sex | 25.02 | 3 | <0.001 |
| Depth | 20.92 | 4 | <0.001 |

**Model 2 (1996 port sampling of commercial catch)**

logit( incidence) = intercept + area + dead or alive

| Coefficient | Value |
|---|---|
| Intercept | –2.744489 |
| Area | –0.667367 |
| Dead or alive | –0.300833 |

Significance tests of main effects

| Effect | Likelihood ratio | df | Probability |
|---|---|---|---|
| Area | 22.39 | 1 | <0.001 |
| Dead or alive | 3.96 | 1 | 0.046 |

carrying uneyed embryos (Table 4). As crab embryos became more developed, so too did the snailfish embryos. Thus, crabs with eyed embryos were not found with uneyed snailfish embryos, and crabs with hatched embryos were not found with uneyed or eyed snailfish embryos. Considering the three development stages of both species as an ordered random variable, the crab developmental stages were highly correlated (rho=0.68, $P$=0.005) with the snailfish developmental stages.

## Genetic determination of species identity

All 14 of the fish samples, but only 10 of the 23 egg and larvae samples, collected by commercial fishery observers were sufficiently well preserved to allow

**Table 3**

Incidence of snailfish (*Careproctus* spp.) egg clusters within golden king crab (*Lithodes aequispinus*). Included are the total landed catch in numbers of live and dead crab, number of crab in each category examined, and the number with egg masses.

| Sampling type | Area | Condition | Catch | Crabs examined | Crabs with fish eggs | Percent infested |
|---|---|---|---|---|---|---|
| Incidence in male crabs >135 mm CL | | | | | | |
| 1996 port | Bering Sea | live | 12788 | 302 | 25 | 8.3 |
| | | dead | 351 | 44 | 7 | 15.9 |
| | Aleutian Is. | live | 46206 | 603 | 15 | 2.5 |
| | | dead | 1508 | 343 | 14 | 4.1 |
| 1982 survey | Bering Sea | live | | 55 | 24 | 43.6 |
| Incidence in female crabs and male crabs ≤135 mm CL | | | | | | |
| 1996 at-sea | Aleutian Is. | male | | 1641 | 12 | 0.7 |
| | | female | | 1631 | 4 | 0.2 |
| 1982 survey | Bering Sea | male | | 183 | 44 | 24.0 |
| | | female | | 274 | 26 | 9.5 |

DNA amplification. *Careproctus furcellus*, *C. rastrinus*, and *C. cypselurus* fish samples could be readily distinguished in the RFLP analysis. Of the 10 usable egg and larvae samples, two were identified as *C. furcellus* and eight clearly differed from all of the fish samples.

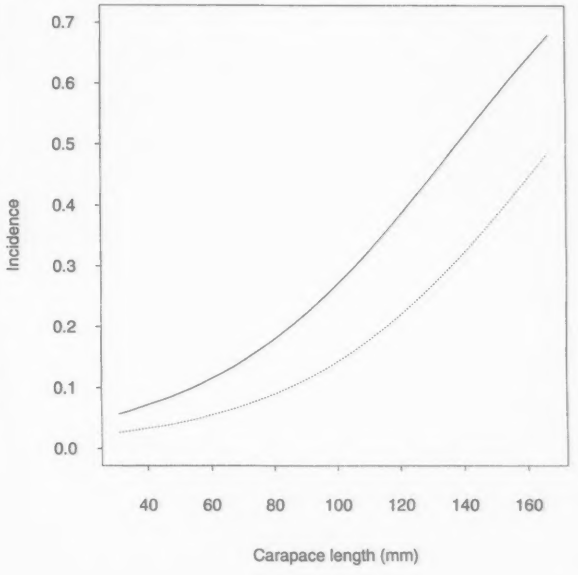


**Figure 9**

Incidence of *Careproctus* eggs and larvae, predicted by the generalized linear model, for male (solid line) and female (dotted line) golden king crab as a function of carapace length.

## Effect of the egg masses on gill function

The presence of egg masses within the branchial chamber of a crab was associated with three distinct pathological conditions of the gills. First, egg masses compressed the gills so strongly that a distinct impression of the egg mass was left on the gill surface (Fig. 10, upper, p. 880). Second, gills on the infested side of a golden king crab were often darker in color than those on the opposing side (Fig 10, lower, p. 880). Third, gills on the infested side often had areas of blackened, necrotic tissue (Fig. 10, p. 881). Several gills with necrotic tissue were examined microscopically after standard histological preparation.[3] A stage-wise progression of tissue damage was evident, beginning as small lesions with hemocyte encapsulations. As the lesions increased in size, they were more likely to be melanized, indicating a chronic condition, and often occluded one or more gill lamellae. Small (occluding one or more lamellae) to medium size (occluding the gill stem) lesions typically possessed new cuticle and normal respiratory epithelium underneath the affected area, indicating that the gill tissue would be regenerated during the next molt. In extreme cases, all of the gills on the affected side of the body were reduced to blackened stubs (Fig. 10, p. 881).

[3] Morado, F. 1996. Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA. Personal commun.

**Figure 10**

Pathological conditions associated with the presence of a snailfish egg mass in the branchial chamber of a golden king crab. Compression of the left gills and impression of individual eggs on the gill surface (upper). Blackened necrotic tissue on the right gills (lower). Complete loss of left gills (facing page). The fifth pereiopods, which act as gill cleaning appendages, are shown in their folded state at the posterior junction of the carapace and abdomen (lower).

## Discussion

The branchial chambers of golden king crab provide *Careproctus* embryos and larvae with a well aerated environment and protection from predators. This advantage would be jeopardized if they were ejected prematurely by crab molting. To minimize the risk of this happening, snailfish must either have the

**Figure 10 (continued)**

ability to choose host crabs that are early in their molt cycle or that have embryonic and larval residence times that are short in relation to the intermolt period of the crab. Evidence that snailfish have both attributes is provided by the association that we found between the embryonic developmental stages of crabs and those of the snailfish found within the crabs.

Preference for newly molted crabs is indicated by the observed presence of young (uneyed) snailfish embryos in female golden king crab carrying uneyed embryos but not in those carrying eyed embryos or remnants of hatched larvae (Table 4), because king crabs deposit their eggs soon after molting. Snailfish may locate newly molted crabs olfactorally, by detecting minute traces of molting hormones in the same way that some male crabs locate premolt females (Ryan, 1966).

A relatively short embryonic and larval residence time, compared with the intermolt period of the crab, is indicated by the presence of late snailfish developmental stages (eyed embryos and larvae) in newly molted (uneyed) female golden king crab (Table 4). The length of the embryonic periods for pink and red snailfish are unknown, but the large size of the eggs and low temperatures of the water in which they incubate (average bottom temperature on the 1982 survey was 3.8°C) indicate that the embryonic periods are probably quite long. Incubation times are greater in colder water and for fish with larger eggs (Pauley and Pullin, 1988), but there are no studies applicable to a large egg-producing deep slope species such as *Careproctus*. If chinook salmon (*Oncorhynchus tshawytscha)* are used as an approximate model for a relatively large egg, cold water species, then 4.5 months would be required for hatching at 3.8°C. (Alderdice and Velsen, 1978). Although this rate of embryonic development may be long for a fish, it is considerably shorter than that of golden king crab which perhaps exceeds 1 year (Somerton and Otto, 1986).

If snailfish can preferentially choose newly molted crabs as hosts, then their spawning season must occur at the same time as the molting season of the

**Table 4**

Number of female golden king crab observed categorized by the development stage of their own embryos and the snailfish embryos within their branchial chambers.

| | Snailfish stages | | |
|---|---|---|---|
| Crab stages | Uneyed | Eyed | Larvae |
| Uneyed | 9 | 4 | 1 |
| Eyed | 0 | 1 | 0 |
| Hatched | 0 | 0 | 3 |

crab. However, the variety of snailfish embryonic stages that occurred during the 1982 sampling (Table 1), coupled with the probable slow developmental rate, indicates that pink and red snailfish have either a protracted spawning season, or perhaps lack spawning seasonality. Previous studies of snailfish reproduction have reported that aseasonal spawning was typical of abyssal and slope snailfishes, although *C. malanurus*, a close relative to pink snailfish, has a seasonal peak in spawning off Oregon (Stein, 1980). The likelihood for preferential selection of early molt-stage crabs is not necessarily diminished by aseasonal spawning in red and pink snailfish because golden king crab also have aseasonal reproduction and molting (Somerton and Otto, 1986).

The incidence of snailfish eggs and larvae was greater in male than in female crabs and increased with crab size in both the 1982 survey (Table 2; Fig. 9) and the 1996 commercial sampling (Table 3). The preference for male crabs as hosts over females is quite pronounced. For example, at the median 1982 sampling depth, a 100-mm male is 1.9 times more likely to contain eggs and larvae than an equal-size female (Fig. 9). As in our study, male *Lithodes tropicalis* had a higher incidence of snailfish eggs than females (Melville-Smith and Louw, 1986), but the apparent sex selection was attributed to size selection and to a large sexual dimorphism in crab size. In our case, we believe sex itself is important in host choice because sex was a significant predictor of incidence even when size and depth effects were included in the model. Such sex selection may not be universal, however, because a previous study of *L. aequispinus* reported that incidence was higher in females (Love and Shirley, 1993). Why a preference for males should occur is not obvious. One possible explanation, based on aquarium observations (Love and Shirley, 1993), is that female golden king crab aggressively defend the embryos attached to their pleopods. Perhaps this aggressiveness can discourage the attempts of a snailfish to extrude her eggs into the branchial chambers of the crab.

The apparent preference for large crabs is also quite pronounced. For example, at the median 1982 sampling depth, a 140-mm-CL male is 1.9 times more likely to contain snailfish eggs or larvae than a 100-mm-CL male (Fig. 9). The apparent preference of snailfish for large crabs is likely due to two distinct attributes associated with size in lithodid crabs. First, large king crabs molt less frequently than small king crabs. Although the molting frequency of golden king crabs is unknown, for male red king crabs (*Paralithodes camtschaticus*), molting frequency diminishes continuously with increasing age (McCaughran and Powell, 1977). Since a lower molt frequency would result in a lower probability of premature release of eggs and larvae, it is an advantageous feature for a perspective host to have. Second, larger crabs have larger branchial chambers to contain snailfish eggs. In our case, egg mass volume increased with branchial volume (Fig. 10), indicating that the size of an egg mass is limited by the size of the branchial chamber. This increase, however, diminished with crab size, indicating that in large crabs the size of an egg mass may be determined more by snailfish fecundity than by the availability of space. It is not clear what effect the space limitation would have on the searching behavior of snailfish. If a female snailfish is capable of partitioning a batch of ripe eggs among several spawning events, then she might be able to reduce the problem of space limitation by depositing eggs in several crabs. If instead female snailfish must deposit their entire batch in one spawning event, then considerably more searching would be required to find a crab with sufficient volume. To help provide some indication of whether a female partitions a batch of eggs, we measured egg batch volume for a single 40-cm pink snailfish, considering only eggs that were free in the ovarian lumen. Because the measured volume (48 mL) is about equal to the median volume of the egg masses found in crabs (Fig. 7), it is possible that a female spawns an entire batch in one event. If this is true, then a female would have to determine, perhaps by probing with her ovipositor, whether a prospective host has sufficient branchial volume for her batch of eggs.

Besides size and sex, other aspects of crab host choice by snailfish have been postulated. Melville-Smith and Louw (1987) suggested that *Careproctus* might preferentially choose only one side of host *L. tropicalus* to deposit egg masses. In our case, neither pink snailfish nor red snailfish chose one side of the crab over the other (binomial test, red snailfish, $P$=0.39, pink snailfish, $P$=0.76). Love and Shirley (1993) and Melville-Smith and Louw (1987) suggested, after finding no crabs with egg and larvae masses in both branchial chambers, that snailfish are inhibited from depositing egg masses in the unaffected branchial chamber of previously infested crabs, presumably to reduce host mortality. In our case, this feature was not true because of 23 crabs that had at least two egg or larvae masses, 13 had masses on both sides.

Because large crabs occur in shallower waters than small crabs and males occur in shallower waters than females, the preferred hosts of both snailfish species occur in the shallower portion of the 1982 depth range. Pink snailfish occur at approximately the same depths as their preferred hosts, but red

snailfish live in deeper waters and must migrate into shallower water to find suitable hosts. For red snailfish, a spawning migration is apparent by the shallower peak in the depth distribution of its egg masses (350 m; Fig. 8) compared with the depth distribution of the fish themselves (650 m; Fig. 5). For pink snailfish, by comparison, the depth distributions of the egg masses and fish nearly coincide. If the spawning migration is undertaken only by ripe fish, then a difference in depth distribution between ripe and unripe fish would be expected for red snailfish but not for pink snailfish. We are unable to account for our observation that the expected shift in depth distribution was found for pink snailfish but not red snailfish.

One shortcoming of the 1982 sampling of snailfish eggs and larvae is that the species identification was based on an apparent species difference in egg and larvae size. Although larvae were collected in an attempt to establish identification by linking larval characteristics to adult fish, the larvae were poorly ossified and their identities could not be determined.[1] In a second attempt at species identification, we subjected the eggs and larvae collected in the 1996 sampling to RFLP analysis. On this basis, it was possible to establish that pink snailfish do deposit eggs in golden king crab. Unfortunately, we had no red snailfish tissue available to establish an identification pattern for the RFLP analysis because red snailfish were not captured during the 1996 sampling (the commercial crab fishery does not extend into sufficiently deep water). Therefore, it was not possible to determine if the 8 of 10 specimens not matching any of the three sampled *Careproctus* species were, as we suspect, red snailfish.

Damage to the gills from the presence of egg masses may result from two causes. First, gill compression could be strong enough to restrict blood flow, resulting in localized necrosis. Second, egg masses could interfere with the functioning of the fifth pereiopod (Fig. 10), which is covered with setae and functions as a gill-cleaning appendage (Pohle, 1989). Experiments with other lithodid crabs have demonstrated that gill fouling similar to the discoloration observed in golden king crab could be experimentally produced either by restricting the movement of the fifth pereiopod or removing the setae from this appendage. The fouling is due not only to the accumulation of detritus on the gill surface, but also the accumulation of a host of organisms that feed on the detritus or directly on the gill tissue itself (Pohle, 1989). In golden king crabs, the degeneration of the gill tissue can proceed to the point that all of the gill tissue on the infested side is missing (Fig 10). In cases of small to medium lesions, it was clear from histological examinations that gill regeneration would occur at the next molt, but crabs with extreme cases of gill degeneration were not examined histologically and it is not known whether gill regeneration would occur. However, in at least one case, a newly molted golden king crab was encountered with no gills on one side of the body. Perhaps, this crab was an extreme case of gill damage which did not regenerate. Gill damage not only influences a crab's respiration but also its capability for ion exchange. Lithodid crabs that died as the result of the restriction of their cleaning appendages displayed considerable abdominal swelling which is indicative of ion imbalance (Pohle, 1989).

The 35% higher mortality of infested male crabs, compared with uninfested crabs, within the live tanks of commercial vessels (Table 4) indicates that the presence of egg masses hinders the ability of crabs to withstand the stress of capture and confinement. The impact on the fishery, however, depends on the incidence of snailfish eggs and larvae as well as the additional mortality they induce. In the case of the 1996 incidence in the Bering Sea (8.5%, Table 3, corrected for deadloss), for example, live-tank mortality increased by only 0.08%. Even at the higher incidence found in 1982 (44%), additional live-tank mortality would have been only 0.40%. Although the mortality that is induced on wild crabs is unknown, it appears that the impact of snailfish parasitism on the golden king crab fishery is small.

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## Literature cited

**Alderdice, D. F., and F. P. J. Velsen.**
**1978.** Relation between temperature and incubation time for eggs of chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 35:69–75.

**Allen J. M., and G. B. Smith.**
**1988.** Atlas and zoography of common fishes in the Bering Sea and northeastern Pacific. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 66, 151 p.

**Anderson, E. M., and G. M. Cailliet.**
**1974.** Crab and snailfish commensalism in Monterey Bay. Underwater Nat. 8:29–31.

**Balbontín, F., I. G. Campodónico, and L. M. Guzmán.**
**1979.** Descripción de huevos y larvae de especies de

*Careproctus* (Pisces: Liparidae) comensales de *Paralomis granulosa* y *Lithodes antarctica* (Crustacea: Lithodidae). An. Inst. Patagonia 10:235–243.

**Dowling, T. E., C. Moritz, J. D. Palmer, and L. H. Rieseberg.**
**1996.** Nucleic acids III: analysis of fragments and restriction sites. *In* D. M. Hillis, C. Moritz, and B. K. Mable (eds.), Molecular systematics, 2nd ed., p. 249–320. Sinauer, MA.

**Hunter, C. J.**
**1969.** Confirmation of symbiotic relationship between liparid fishes (*Careproctus* spp.) and male king crab (*Paralithodes camtschatica*). Pac. Sci. 23:546–547.

**Kido, K.**
**1988.** Phylogeny of the family Liparididae, with the taxonomy of the species found around Japan. Mem. Fac. Fish. Hokkaido Univ. 35:125–256.

**Love, D. C., and T. C. Shirley.**
**1993.** Parasitism of the golden king crab, *Lithodes aequispinus* Benedict, 1895 (Decapoda, Anomura, Lithodidae) by a liparid fish. Crustaceana 65:97–104.

**McCaughran, D. A., and G. C. Powell.**
**1977.** Growth model for Alaskan king crab (*Paralithodes camtschatica*). J. Fish. Res. Board Can. 34:989–995.

**Melville-Smith R., and E. Louw.**
**1987.** An association between a liparid fish species and the stone crab *Lithodes tropicalis* (Decapoda, Anomura). S.-Afr. Tydskr. Dierk. 22:140–142.

**Parrish, R.**
**1972.** Symbiosis in the blacktail snailfish, *Careproctus melanurus*, and the box crab *Lopholithodes foraminatus*. Calif. Fish Game 68:239–240.

**Pauly, D., and R. S. V. Pullin.**
**1988.** Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. Environ. Biol. Fishes 22:261–271.

**Peden, A. E., and C. A. Corbett.**
**1973.** Commensalism between a liparid fish, *Careproctus* sp., and the lithodid box crab *Lopholithodes foraminatus*. Can. J. Zool. 51:555–556.

**Pohle, G.**
**1989.** Gill and embryo grooming in lithodid crabs: comparative functional morphology based on *Lithodes maja*. *In* B. E. Felgenhauer, L. Watling, and A. B. Thistle (eds.), Functional morphology of feeding and grooming in crustacea, p 75–94. A. A. Balkema, Rotterdam.

**Powell, G. C., and R. B. Nickerson.**
**1965.** Reproduction of king crabs, *Paralithodes camtschatica* (Tilesius). J. Fish. Res. Board Can. 22:101–111.

**Rass, T. S.**
**1950.** Zamechatel'nyi sluchai biologicheskoi svyazi ryby i kraba [An unusual instance of a biological relation between fish and crabs]. Priroda 39(7):68–69. In Russian. Translated by Paul Macy, U. S. Fish and Wildlife Service, Translation Series No. 41. [Available from the Northwest Fisheries Science Center, 2725 Montlake Blvd. E. Seattle, WA 98102.]

**Ryan, E. P.**
**1966.** Pheromone: evidence in a decapod crustacean. Science 151:340–341.

**Sloan, N. A.**
**1985.** Life history characteristics of fjord-dwelling golden king crab *Lithodes aequispina*. Mar. Ecol. Prog. Ser. 22:219–228.

**Somerton, D. A., and R. S. Otto.**
**1986.** Distribution and reproductive biology of the golden king crab, *Lithodes aequispina*, in the eastern Bering Sea. Fish. Bull. 84:571–584.

**Stein, D. L.**
**1980.** Aspects of reproduction of liparid fishes from the continental slope and abyssal plain off Oregon, with notes on growth. Copeia 1980:687–699.

**Syrjala, S. E.**
**1996.** A statistical test for a difference between the spatial distributions of two populations. Ecology 77:75–80.

**Venables, W. N., and B. D. Ripley.**
**1994.** Modern applied statistics with S-plus. Springer-Verlag , New York, NY, 462 p.

**Vinogradov, K. A.**
**1950.** K biologii Tikhookeanskogo pinagora v Kamchatskikh vodakh [Biology of the Pacific lumpfishes in Kamchatka waters]. Priroda (Leningrad) 39(7):69–70.

# Settlement and recruitment of queen conch, *Strombus gigas*, in seagrass meadows: associations with habitat and micropredators

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**Abstract.**—A suction dredge survey was conducted in the Bahamas in a tidal flow field system which contained a nursery ground for the economically significant gastropod *Strombus gigas* (queen conch). Settlement of larval conch within the system was associated with the specific location of the nursery and positively correlated with subsequent recruitment to the juvenile population (<45 mm shell length). Settlement was relatively independent of habitat features including depth, sediment characteristics, and macrophytes. Conversely, densities of micropredators (small crabs, shrimp, and predaceous gastropods) capable of consuming early postsettlement conch were often correlated with habitat features such as seagrass shoot density, seagrass detritus, and organic content of the sediment. The density of small xanthid crabs (mode=1.5 mm carapace width) was positively correlated with density of live postsettlement conch (mean $\leq 4/m^2$), suggesting that conch settle in predator-prone areas or that the crabs respond numerically to small conch (or both). Densities of xanthids were very high (to $>200/m^2$), and the crabs probably represent an important source of mortality for small conch in the primary nursery ground. Shells of dead conch indicated that molluscan and asteroid predators probably caused most of the predatory mortality on young conch that settled outside the nursery. Because critical settlement and recruitment habitats for queen conch are associated with particular hydrographic conditions, these habitats cannot be identified or predicted simply by mapping obvious features such as seagrass cover, depth, or sediment type. An understanding of dynamic processes, such as larval transport and retention, selective settlement mechanisms, and trophic ecology, will be required to identify critical nursery habitats.

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Distribution of marine species with planktonic larvae is a function of both pre- and postsettlement processes. Location of reproductive sources, physical and oceanographic processes, duration and survivorship of larval stages, and larval behavior combine to determine settlement location, whereas predation, suitable habitat, and the behavior of early juveniles affect the number of individuals that survive to represent juvenile recruitment (Keough and Downes, 1982; Luckenbach, 1984; Connell, 1985). Experiments conducted in the laboratory with a large variety of species have shown that settlement in the field is probably a nonrandom process for the majority of marine invertebrates, and settlement behavior is usually assumed to place juveniles in habitats most suitable for growth or survival (or both) (Hadfield and Scheuer, 1985; Butman and Grassle, 1992; Grassle et al., 1992; Davis and Stoner, 1994; Stoner et al., 1996a).

Despite an abundance of data and experiments on settlement, relatively little is known about the relative importance of settlement rate and postsettlement predation in controlling local recruitment of most species to juvenile stages (Osman and Whitlatch, 1995). Because early postsettlement juveniles of many benthic species, especially those associated with soft sediments, are difficult to survey, quantitative data related to recruitment often depend upon observations of the smallest juveniles that can be detected easily. The result is a relatively poor understanding of the crucial period between settlement and first detection in the benthos (Keough and Downes, 1982; Luckenbach, 1984).

Queen conch (*Strombus gigas*) is a large gastropod that has great economic significance throughout the Caribbean and adjacent regions (Appeldoorn, 1994). Despite the wealth of information now available for conch larvae (Davis, 1994; Stoner and Davis, 1997), juveniles in year class 1 (e.g. Marshall, 1992; Iversen et al., 1987; Appeldoorn, 1994), and adults (Appeldoorn, 1988; Stoner and Sandt, 1992), little is known about larval settlement and the early postsettlement stage. Newly settled queen conch (<10 mm) are cryptic, often buried (Iversen et al., 1989; Sandt and Stoner, 1993), and are readily preyed upon by micropredators such as xanthid

crabs and certain polychaetes (Ray-Culp et al., 1997). Queen conch <50 mm shell length have rarely been encountered in the field (Iversen et al., 1994; Ray and Stoner, 1995; Iversen and Jory, 1997), and the only density data extant for these small conch were collected by Sandt and Stoner (1993) near Lee Stocking Island, Bahamas. However, queen conch settle at a large size (1.2 mm), compared with many other mollusks, and even the smallest juvenile, unless crushed completely, leaves an identifiable shell record of its settlement in the sediment (our study). This record can be used to examine the settlement-recruitment relationship and spatial variation in settlement.

Our study had three primary objectives. First, we conducted an extensive dredge survey for newly settled queen conch in and around a well-studied nursery area in the central Bahamas during the summer recruitment season in 1992. The survey was designed to test the hypothesis that the long-term distribution pattern of year-class 1 and 2 conch was associated with settlement pattern. The sum of live and dead individuals was used as an index of settlement at each sampling station, and the number of surviving (live) conch served as an index of recruitment. Through this survey, we gathered the first quantitative data on the distribution of queen conch <45 mm shell length (1.5–44 mm). Second, we used observations of shell damage sustained by dead individuals to determine the primary predatory forms on newly settled queen conch. We also examined spatial variation in predation type. Third, we examined the effects of environmental characteristics, such as depth, sediments, macrophytes, older conspecifics, and distribution of potential predators, on the observed distribution of newly settled queen conch.

## Study area

The Exuma Cays are an important source of queen conch for a large fishery in the Bahamas; the island chain is 250 km long, bordered to the east by the Exuma Sound, and to the west by the Great Bahama Bank. Water exchange occurs through numerous tidal inlets separating the islands, creating extensive tidal flow fields on the shallow bank. Nurseries of juvenile conch (1- and 2-yr-old, 70–150 mm SL) are located primarily on the bank side of the Exuma Cays near these inlets and are typically associated with seagrass meadows (2–4 m deep) that are flushed with clear, oligotrophic water from the Sound during flood tide (Stoner et al., 1996b).

We elected to survey postlarval conch in a tidal flow field located west of Lee Stocking Island and south of Norman's Pond Cay because the conch nursery near Shark Rock (Fig. 1) is well studied. Prior to sampling, five years of data had been collected on the distribution and abundance of 1- and 2-year-old juvenile conch, and environmental characteristics such as seagrass biomass, depth, and tidal currents had been mapped (Jones, 1996; Stoner et al., 1996b). Drogue studies have shown that, on flood tide, water from the Sound enters the inlet north of Lee Stocking Island, passes close to Shark Rock, and flows west of the nursery for a distance that is dependent on wind conditions and tidal phase (e.g. spring, neap) (Stoner et al., 1994). Tidal currents, which reach 100 cm/s at midtide, flow between sand bars in an S-shaped pattern following the bank bathymetry (Fig. 1B). The middle of the tidal channel is ~3 m deep and vegetated primarily with the seagrass *Thalassia testudinum*. Depth and seagrass density gradually decrease from midchannel to bare sand on both sides of the channel. Tidal range is ~1 m.

Annual surveys conducted between 1988 and 1992 showed that aggregations of juvenile queen conch always grazed within a 2-km long section of the tidal flow field close to Shark Rock (Fig. 1) and, at any given time, occupied only portions of the suitable habitat available (Stoner et al., 1996b). Stations for this study were established with reference to long-term distribution of conch in this area (Stoner and Waite, 1990; Stoner and Ray, 1993; Ray and Stoner, 1994) and to represent both down-channel and across-channel dimensions of the flow field. One line of stations was established in midchannel down the flow field from Adderly Cay (A) to Cook's Cay (F) with an attempt to locate all of the stations in similar depth and moderate seagrass shoot density. Stations A and F were each located ~4 km from the geographic center of the traditional nursery ground (Fig. 1), and stations B and E were located ~250 m outside the northeastern and southwestern ends of the nursery, respectively. Stations C3 and D3 were each established ~500 m from the geographic center of the nursery (Fig. 1C).

To represent the across-flow field dimension, five more stations were selected along two transects that lay perpendicular to the main axis of the tidal current at stations C3 and D3, as well as across the seagrass gradient. *Thalassia testudinum* density ranged from 4 to 704 shoots/$m^2$ and from 0.5 to 178 g dry wt/$m^2$ across this gradient in 1991 (Ray and Stoner, 1994) (Fig. 1C). Three stations were established along the D transect in addition to D3, increasing in macrophyte cover from bare sand at D1 to high seagrass and detrital biomass at D4. Along transect C, two stations were established in addition to C3, ranging from sand to moderate seagrass shoot density and macrophyte biomass. There were no areas of high seagrass biomass present near transect C and,

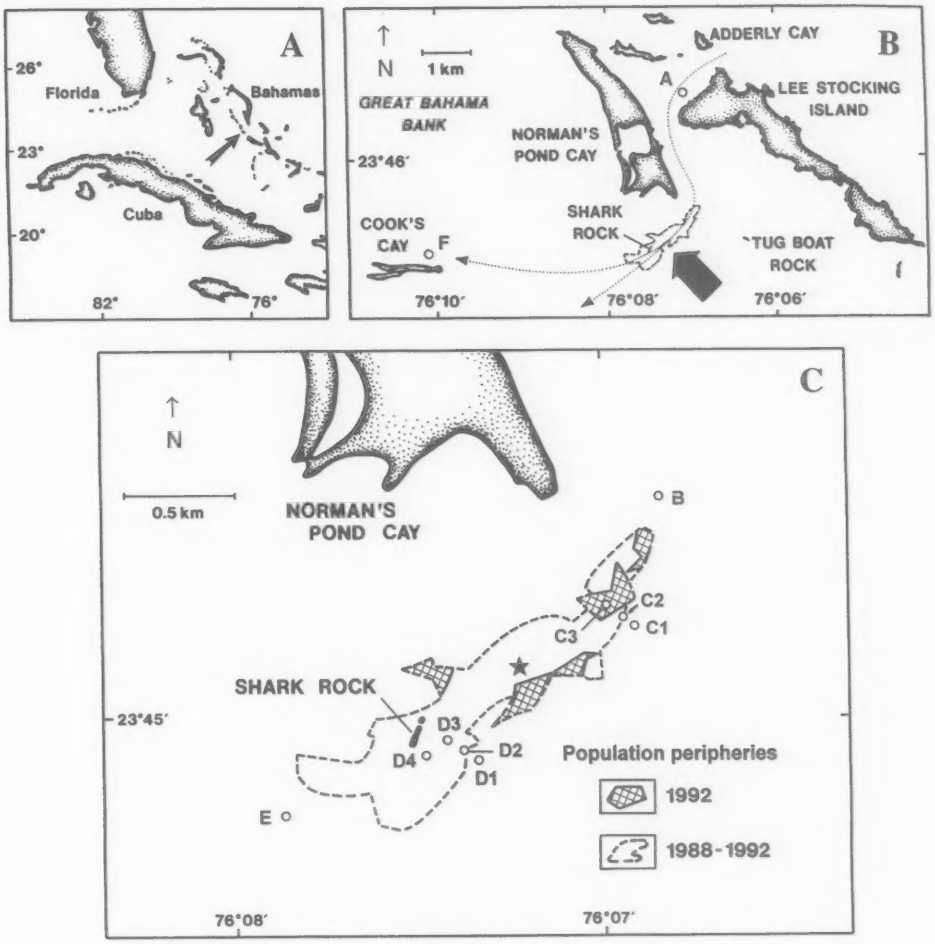


**Figure 1**

(**A**) Study area in the southern Exuma Cays, central Bahamas. (**B**) On the flood tide, water from Exuma Sound passes between Adderly Cay and Lee Stocking Island, flowing in an S-shaped pattern past Shark Rock towards Cook's Cay (dotted arrow). Stations A and F represented ends of the flow field. (**C**) Nine more stations were selected with respect to the long-term (1988–92) location of the juvenile queen conch aggregation, delineated by dashed line. In July 1992, just before dredge sampling was conducted, the aggregation occupied the areas shown in hatched polygons. Star indicates center of aggregation.

consequently, no station was directly comparable to D4. Other environmental characteristics, such as depth and certain sediment characteristics, also varied across the flow field (see "Results" section, Tables 1 and 2).

## Methods

### Dredge sampling

Given that maximum densities of conch veligers are observed during midsummer (June through August) and that the larval period lasts 3–4 weeks (Davis et al., 1993), dredge sampling for newly settled conch was conducted at the end of the summer, 24 August to 1 September 1992. Scuba equipment was used for all underwater work. At each of the 11 stations, water depth was measured and corrected to mean low water (MLW), and duplicate sediment samples were collected with a PVC core (diameter=40 mm, depth=5 cm) prior to dredging.

Sediment samples were rinsed with freshwater and dried at 80°C to constant weight. A subsample (~15–20 g) was incinerated at 550°C in a muffle furnace

for 4 h to determine organic content, calculated as the percent difference between dry weight and ash-free dry weight. A second rinsed subsample (~20 g) was analyzed by using standard dry sieve procedures (Folk, 1966), and product moment statistics were used to calculate mean grain size (McBride, 1971). The silt-clay fraction (>4.0 ø, <62 mm), always < 9% of sediment dry weight, was not fractionated.

Replicate dredge sample plots ($n$=5 for all stations, except C3, where $n$=6) were delineated by a circular enclosure made of aluminum sheet metal (area=0.5 $m^2$, height=0.3 m) that was placed haphazardly at each station. The enclosure was pushed securely into the sediment to prevent escape of motile fauna. In the middle of each sample plot, the number of *Thalassia testudinum* shoots was counted in a quadrat (25 × 25 cm). A gasoline-powered suction dredge (modified from Brook, 1979) was used to collect year-class 0 queen conch, other macrofauna, and associated macrophytes from each plot. The dredge created high-pressure water flow which, as a result of Venturi principle, drew algae, detritus, sediments, and macrofauna through a PVC intake tube (diameter=7.6 cm) into a mesh bag (40 × 70 cm, 1.2 mm mesh). Preliminary sampling in the study area and observations of year-class 0 conch (<45 mm SL) indicated that conch in the nursery ground buried no deeper than 5 cm into the sediment. Therefore, sediments from plots with seagrass were removed to the depth at which *T. testudinum* rhizomes occurred (usually 8–15 cm); in bare sand, dredging was 8–10 cm deep. Unlike the other macrophytes, living seagrass did not detach easily and was not collected with the dredge material. The mesh bags holding the dredged materials were tied securely underwater and later fixed in a 10% formalin-seawater mixture containing rose bengal as a staining agent. After 24 hours, each sample was rinsed onto a sieve (1.2 mm) and preserved in 70% ethanol until sorted.

Macrophytes were sorted into three components: *T. testudinum* detritus (senescent blades and fragments), the green macroalga *Batophora oerstedi*, and the red algae *Laurencia* spp. Occasionally fronds of calcareous green algae (including *Halimeda* spp., *Penicillus capitatus*, *Udotea* spp., and *Rhipocephalus phoenix*) were collected, but they were sparsely distributed and not quantified. Each fraction was rinsed with freshwater to remove salts and dried at 80°C to constant weight (~24 h) so that biomass (g dry wt/ $m^2$) could be calculated.

For ease in extracting newly settled queen conch and their potential predators, sediments were divided into two fractions, those retained on 1.2- and 2.0-mm sieves. A conch was classified as alive if its soft tissue and operculum were intact. Shortly after death, the operculum detaches from the foot, and soft tissues decompose quickly. Therefore, dead and living conch were easily distinguished. We do not believe that conch shells were damaged during collection because the dredge apparatus lifted samples off the bottom by suction that could be controlled and the materials collected did not pass through an impellor. Care was also taken so that the fauna were not damaged in sieving. None of the conch classified as alive at collection had damaged shells, and most other taxa, such as polychaetes and crabs, were in good (i.e. whole) condition.

To gain insight into modes of predation, shells of dead conch were classified as 1) whole and undamaged, 2) drilled, 3) peeled back along the spire line, or 4) crushed. Whole shells of dead conch were attributed to predation by mollusks or asteroids (Jory, 1982; Iversen et al., 1986; Ray and Stoner, 1995), drilled shells were probably the result of mollusk kills (Vermeij, 1987), and peeled and crushed shells were attributed to crustaceans (Randall, 1964; Vermeij, 1982, 1987; Davis, 1992). Whole shells can also result from nonpredatory mortality. The proportion of dead individuals was used as an indicator of post-settlement mortality.

Whole shells (from both live and dead conch) were measured for shell length. When only a shell spire or shell aperture was found, total shell length for the dead animal was calculated on the basis of regression formulae derived from measurements of 20 whole shells ranging in size from 3 to 40 mm total length:

$$Length = (spire\ length \times 2.4) - 1.8; \qquad [r^2=0.991]$$

$$Length = (aperture\ length \times 1.6) + 0.5. \qquad [r^2=0.998]$$

Given that queen conch settle into nursery grounds during a distinct season, it was possible to determine if an individual had indeed settled in 1992 on the basis of its size, color, and the amount of biological encrustation. Settlement of queen conch can occur at the beginning of May, and growth rates during the early postsettlement period may be as great as 0.45 mm/d (Ray and Stoner, 1994); therefore, we considered conch ≤45 mm in total shell length to be members of year-class 0. Also, conch shells lose their pink interior color within a few weeks after death. Animals that settled in 1992 were easily discerned on the basis of shell size and color.

Xanthid and portunid crabs and alpheid shrimps were extracted from samples because they were abundant and known to be significant predators of postsettlement conch (Ray-Culp et al., 1997). Olivid and marginellid gastropods were also removed and counted as potential predators. Carapace width (includ-

ing lateral spines) of crabs was measured, carapace length for shrimps, and shell length of gastropods.

### Data analysis

To discern station differences, density data from the dredge sampling were log-transformed ($\log_{10}(n+1)$) to improve homogeneity of variance (Cochran's test, $P>0.05$) and analysed by using 1-way ANOVA following the guidelines of Day and Quinn (1989). Tukey's multiple comparison test was performed to determine pairwise relationships. Seven measures of density were examined: live conch, dead conch, total conch, alpheids, portunids, xanthids, and total predators.

Relationships between these seven density measures and eight environmental variables (water depth, distance of the station from the center of juvenile queen conch aggregation, sediment grain size, sediment organics, *Thalassia testudinum* shoot density, weight of *T. testudinum* detritus, and biomass of the macroalgae *Batophora oerstedi* and *Laurencia* spp.) were examined with pairwise correlation. Log-transformation improved the relationships, and Pearson correlation coefficients are reported for the log-transformed variables.

The relationships between conch density (live, dead, and total) and each of the five predator families were also examined with correlation. Variables were not transformed in the analysis because transformation did not improve the correlations.

## Results

### Habitat characteristics

Depth down the flow field was relatively uniform, ranging from 2.8 to 3.3 m at MLW, except at E where depth was 2 m (Table 1). Across the flow field at transect D, depth increased from bare sand (1.3 m) to high seagrass density (3.3 m), and, at transect C, greatest depth (3.5 m) occurred in low density seagrass (C2). Sediments were fine to medium sands (1.4–2.6 ø), with mean grain size decreasing slightly (increasing ø) with depth over both transects C and D (Table 1). Organic content of the sediments across transect D also increased with depth, ranging from 2.7% (station D1) to 5.2% (station D4). The range of organic content in the down flow-field dimension was 3.0–4.6%.

*Thalassia testudinum* shoot density decreased down the flow field from 784 to 320 shoots/m$^2$ (Table 2). Across the channel, shoot density increased rapidly from 0 to >500 shoots/m$^2$ in both transects, as had been in-

**Table 1**

Habitat characteristics for 11 dredge stations in the Shark Rock flow field. Depth was at mean low water and distance was measured from each station to the center of the juvenile queen conch nursery (see Fig. 1). Means and the range of values (parentheses) are given for sediment grain size and organics ($n$=2 for all stations except C3, where $n$=3). Data for stations C3 and D3 are given twice for ease of comparison in both flow-field dimensions.

| | | | Sediments | |
|---|---|---|---|---|
| Station | Depth (m) | Distance (km) | Grain size (ø) | Organics (% dry wt) |
| Down flow field, midchannel | | | | |
| A | 2.8 | 3.60 | 2.57 (2.56–2.58) | 4.08 (3.68–4.50) |
| B | 3.0 | 1.00 | 1.81 (1.78–1.84) | 4.05 (3.46–4.65) |
| C3 | 3.1 | 0.55 | 1.84 (1.63–1.95) | 3.54 (3.17–3.75) |
| D3 | 2.8 | 0.55 | 2.07 (2.04–2.10) | 4.56 (4.43–4.69) |
| E | 2.0 | 1.10 | 1.89 (1.86–1.92) | 2.95 (2.95–2.95) |
| F | 3.3 | 4.50 | 2.04 (1.97–2.12) | 3.99 (3.98–4.00) |
| Across flow field | | | | |
| Transect C | | | | |
| C1 | 2.0 | 0.70 | 1.51 (1.27–1.74) | 2.18 (2.16–2.21) |
| C2 | 3.5 | 0.70 | 1.78 (1.73–1.83) | 3.31 (3.09–3.53) |
| C3 | 3.1 | 0.55 | 1.84 (1.63–1.95) | 3.54 (3.17–3.75) |
| Transect D | | | | |
| D1 | 1.3 | 0.60 | 1.44 (1.27–1.60) | 2.72 (2.60–2.84) |
| D2 | 2.1 | 0.55 | 1.73 (1.72–1.74) | 3.12 (3.06–3.19) |
| D3 | 2.8 | 0.55 | 2.07 (2.04–2.10) | 4.56 (4.43–4.69) |
| D4 | 3.3 | 0.70 | 1.87 (1.60–2.14) | 5.24 (5.12–5.37) |

**Table 2**

Macrophyte characteristics for 11 dredge stations in the Shark Rock flow field (see Fig. 1). Shoot count and detritus values are for *Thalassia testudinum*. All values are mean ±SE ($n$=5 for all stations except C3, where $n$=6). Data for stations C3 and D3 are given twice for ease of comparison in both flow-field dimensions.

| Station | Shoot density (no./m$^2$) | Biomass (g dry wt/m$^2$) | | |
|---|---|---|---|---|
| | | Detritus | *B. oerstedi* | *Laurencia* spp. |
| Down flow field, midchannel | | | | |
| A | 784 ± 36 | 353 ± 49 | 0.02 ± 0.01 | 0.20 ± 0.05 |
| B | 640 ± 65 | 144 ± 20 | 0.80 ± 0.40 | 0.04 ± 0.03 |
| C3 | 536 ± 26 | 30 ± 11 | 0.007 ± 0.004 | 0.20 ± 0.18 |
| D3 | 528 ± 36 | 139 ± 20 | 24.08 ± 6.00 | 0.84 ± 0.27 |
| E | 352 ± 22 | 29 ± 10 | 44.76 ± 4.77 | 0.66 ± 0.13 |
| F | 320 ± 17 | 34 ± 8 | 2.18 ± 0.57 | 0 ± 0 |
| Across flow field | | | | |
| Transect C | | | | |
| C1 | 0 ± 0 | 0.06 ± 0.04 | 0.02 ± 0.03 | 0 ± 0 |
| C2 | 288 ± 44 | 37 ± 14 | 1.76 ± 0.45 | 0.34 ± 0.18 |
| C3 | 536 ± 26 | 30 ± 11 | 0.007 ± 0.004 | 0.20 ± 0.18 |
| Transect D | | | | |
| D1 | 0 ± 0 | 0.08 ± 0.04 | 0.06 ± 0.04 | 0 ± 0 |
| D2 | 240 ± 36 | 14 ± 4 | 3.68 ± 0.32 | 1.60 ± 0.04 |
| D3 | 528 ± 36 | 139 ± 20 | 24.08 ± 6.00 | 0.84 ± 0.27 |
| D4 | 544 ± 22 | 180 ± 17 | 0.22 ± 0.23 | 1.58 ± 0.83 |

tended in the sampling design. A similar increase occurred with *T. testudinum* detritus, with the exception of a relatively low value at station C3 (Table 2), where the aggregation of year-class 1 and 2 juvenile conch undoubtedly had a grazing effect (Fig. 1). As was intended, shoot density and detritus biomass increased across transect D, and values were highest at station D4. Algal biomass was noticeably high only at stations D3 and E (where standing crops of *Batophora oerstedi* were 24 and 45 g dry wt/m$^2$, respectively), and particularly low at station C3 (grazed by conch). Biomass of *Laurencia* spp. was low at all stations (≤1.6 g dry wt/m$^2$).

## Newly settled conch

Newly settled queen conch were collected in dredge samples at all 11 stations except F (Fig. 2). Down the flow field, mean total density was relatively high (8–12 conch/m$^2$) at stations B, C3, and D3, and low (0–2.4 conch/m$^2$) at stations A, E, and F, although differences were only significant between C3 and F (Fig. 2). There was a significant negative correlation between total conch and distance from the geographic center of the long-term aggregation (Table 3); both total density and density of dead conch decreased with distance from C3 (Fig. 2). All of the conch collected at stations A and E were dead, as were most (60–86%) from the other down flow field stations. Live conch were collected at stations B, C3, and D3, with maximum density (4 conch/m$^2$) observed at B.

Across the flow field, mean total conch density appeared to increase with seagrass density in transect C (Fig. 2), although differences were not significant. Values were relatively uniform at all of the stations in transect D, ranging from 4.0 to 8.4/m$^2$. Live newly settled conch were most abundant at stations C3 (1.7/m$^2$) and D3 (1.6/m$^2$), where seagrass density was moderate. Significant numbers of live individuals were also collected at the other stations with at least some seagrass (C2, D2, D4). However, all of the conch dredged from the bare sand stations (C1, D1) were dead.

When data for 11 stations were included in the analysis, live conch density (the index of recruitment) had a significant positive correlation with total conch density (the index of settlement) (coefficient of correlation [$r$]=0.703, $P$=0.016). The percentage of the total conch that were dead provides an index of mortality. This index was negatively correlated with settlement ($r$=0.654, $P$=0.04).

Live individuals ranged in size from 3.3 mm to 38.5 mm with the mode at 10–14.9 mm (Fig. 3). Dead individuals ranged in shell length from 1.5 to 44 mm with the mode at 1.0–4.9 mm. The percentage of dead conch with whole, undamaged shells decreased across the flow field along both transects in the direction of increasing seagrass density and increasing density of

**Table 3**

Pearson correlation coefficients ($n$=11) for the pairwise relationship between each of seven dependent variables (i.e. newly settled queen conch and predators) and eight independent variables. Depth was at mean low water; distance was measured from station to center of juvenile queen conch aggregation (see Fig. 1); grain size and organics are for sediments; shoot density and detritus values are for *T. testudinum*. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$. All of the dependent variables were transformed ($\log_{10}(x+1)$) prior to analysis.

| | Live conch | Total conch | Xanthids | Alpheids | Portunids | Olivids | Marginellids |
|---|---|---|---|---|---|---|---|
| Depth | 0.474 | 0.123 | 0.710* | 0.428 | –0.081 | 0.063 | 0.231 |
| Distance | –0.393 | –0.770** | 0.141 | 0.358 | –0.158 | 0.165 | –0.346 |
| Grain size | –0.027 | –0.312 | 0.533 | 0.821** | 0.337 | –0.254 | –0.233 |
| Organics | 0.346 | 0.196 | 0.868*** | 0.694* | 0.041 | –0.398 | 0.304 |
| Shoots | 0.454 | 0.168 | 0.812** | 0.924*** | 0.496 | –0.460 | 0.273 |
| Detritus | 0.085 | 0.038 | 0.537 | 0.947*** | 0.515 | –0.707* | 0.103 |
| *B. oerstedi* | –0.106 | –0.260 | 0.120 | –0.091 | –0.013 | 0.164 | –0.020 |
| *Laurencia* spp. | –0.026 | 0.182 | 0.237 | 0.164 | –0.311 | –0.205 | 0.082 |

year-class 1 and 2 conch (Table 4). The pattern of crushed shells was reverse. In the down flow-field dimension, the percentage of whole empty shells was lowest at stations within the nursery ground and at station F. Crushed shells made up the highest proportion of dead, newly settled conch within the nursery area. Overall, few shells were drilled (2.8%) or peeled (13.5%), and most were crushed (56.7%).

**Table 4**

Number of dead newly settled queen conch dredged from 11 stations for each of four shell conditions (see text for definitions). Values are station percentages followed by ($n$). Data for stations C3 and D3 are given twice for ease of comparison in both flow field dimensions.

| Station | Whole | Drilled | Peeled | Crushed | Total |
|---|---|---|---|---|---|
| Down flow field, midchannel | | | | | |
| A | 33.3 (2) | 16.7 (1) | 0 (0) | 50.0 (3) | (6) |
| B | 6.7 (1) | 0 (0) | 13.3 (2) | 80.0 (12) | (15) |
| C3 | 9.7 (3) | 0 (0) | 25.8 (8) | 64.5 (20) | (31) |
| D3 | 18.7 (3) | 0 (0) | 12.5 (2) | 68.8 (11) | (16) |
| E | 33.3 (1) | 0 (0) | 33.3 (1) | 33.3 (1) | (3) |
| F | 0 (0) | 0 (0) | 0 (0) | 0 (0) | (0) |
| Total | 14.1 (10) | 1.4 (1) | 18.3 (13) | 66.2 (47) | (71) |
| Across flow field | | | | | |
| Transect C | | | | | |
| C1 | 57.1 (4) | 0 (0) | 28.6 (2) | 14.3 (1) | (7) |
| C2 | 17.6 (3) | 0 (0) | 5.9 (1) | 76.5 (13) | (17) |
| C3 | 9.7 (3) | 0 (0) | 25.8 (8) | 64.5 (20) | (31) |
| Total | 18.2 (10) | 0 (0) | 20 (11) | 61.8 (34) | (55) |
| Transect D | | | | | |
| D1 | 64.7 (11) | 0 (0) | 5.9 (1) | 29.4 (5) | (17) |
| D2 | 55.6 (5) | 11.1 (1) | 22.2 (2) | 11.1 (1) | (9) |
| D3 | 18.7 (3) | 0 (0) | 12.5 (2) | 68.8 (11) | (16) |
| D4 | 25.0 (5) | 10.0 (2) | 0 (0) | 65.0 (13) | (20) |
| Total | 38.7 (24) | 4.8 (3) | 8.1 (5) | 48.4 (30) | (62) |
| Grand total[1] | 27.0 (38) | 2.8 (4) | 13.5 (19) | 56.7 (80) | (141) |

[1] Stations C3 and D3 are included only once in grand total.

## Conch predators

Xanthid crabs (mostly *Micropanope* spp.) composed the predator group with the greatest densities and governed the density distribution of total predators in both flow-field dimensions (Fig. 4). Xanthids were most abundant (102–286 per $m^2$) at stations B, D3, and D4, and were $\leq 36/m^2$ at all other stations. Alpheid density decreased down the flow field (Fig. 3), increased with seagrass density across the flow field (Figs. 3 and 4), and had a high positive correlation with detritus ($r$=0.947, Table 3). Xanthid and alpheid densities also had high positive correlations with *Thalassia testudinum* shoot density and sediment organics (Table 3). Portunid crab densities were relatively low, compared with the two other crustacean families. They decreased down the flow field from a maximum at A ($7.6/m^2$) to 0 at F (Fig. 4).

Of the two predaceous mollusc families observed, the Olividae were most abundant at stations C2 ($18/m^2$) and F ($25/m^2$) (ANOVA, $F_{10,45}$=5.69, $P$<0.001). Values were $\leq 7.2/m^2$ at all other stations and the data were not plotted. Olivids were negatively correlated with detritus (Table 3). The Marginellidae were most abundant at B ($9.2/m^2$) and D4 ($6.8/m^2$) and densities were $\leq 2/m^2$ at all other stations, but the differences

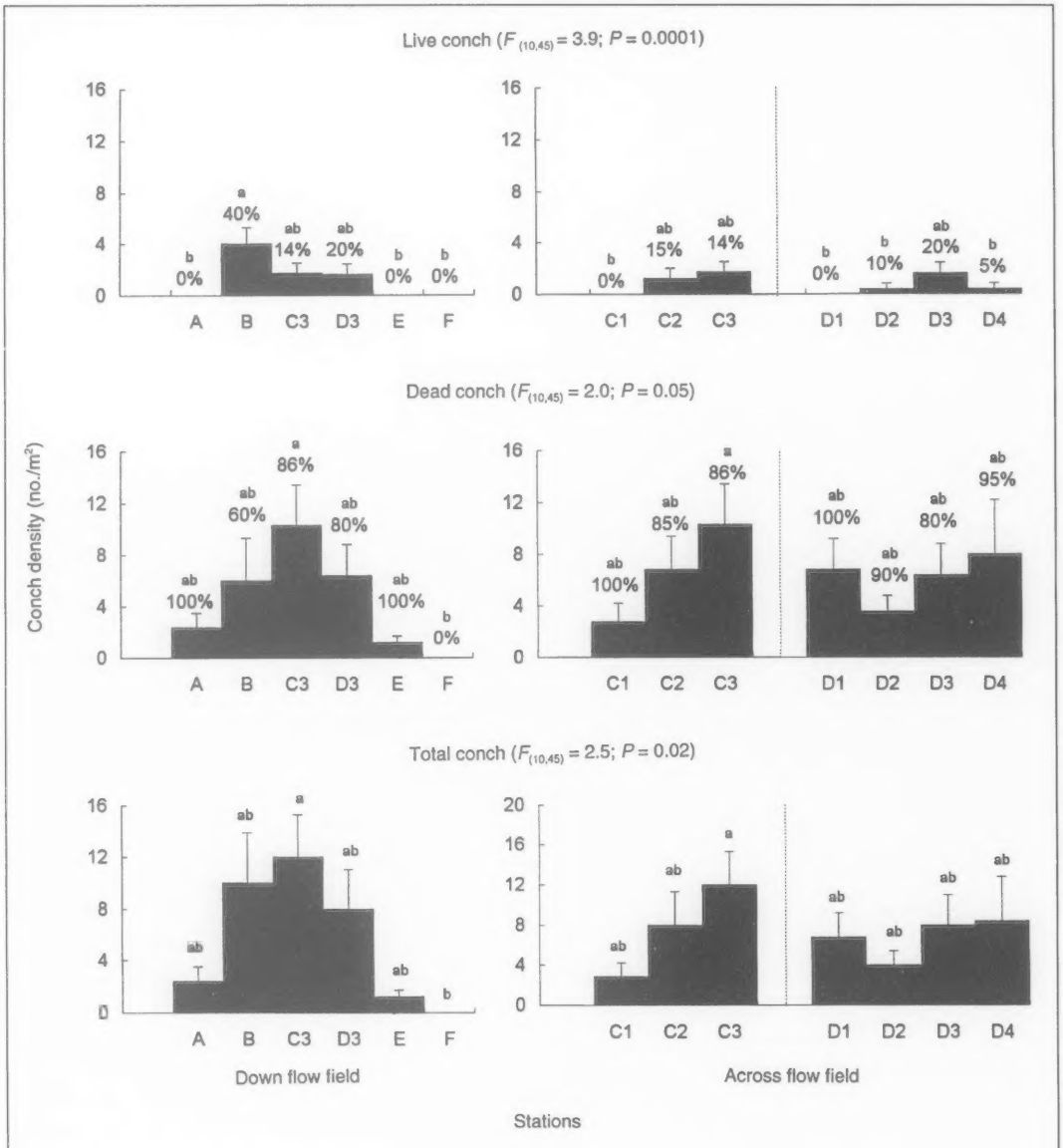
Live conch ($F_{(10,45)}$ = 3.9; $P$ = 0.0001)
Dead conch ($F_{(10,45)}$ = 2.0; $P$ = 0.05)
Total conch ($F_{(10,45)}$ = 2.5; $P$ = 0.02)
Conch density (no./m²)
A B C3 D3 E F
C1 C2 C3 D1 D2 D3 D4
Down flow field
Across flow field
Stations

**Figure 2**

Density of live, dead, and total queen conch collected from 11 dredge stations located down and across the Shark Rock flow field. Stations C3 and D3 are illustrated in both dimensions for comparison. Values are mean ± SE ($n$=5 for all stations except C3, where $n$=6). Percentage of the total count represented by live and dead conch at each station is also given. $F$ and $P$ values are for 1-way ANOVAs made for each variable and flow-field dimension. Means that were not statistically different ($P$>0.05) are designated by similar letters (Tukey multiple comparison test on log-transformed data).

were not significant ($F_{10,45}$=1.92, $P$=0.067). Neither olivids nor marginellids were collected at station A.

Because size-frequency distribution of each predator group varied little among the 11 stations, measurements from all stations were considered together (Table 5). The majority of xanthids were very small, with a mode of just 1.5 mm carapace width. The largest predator collected was a portunid (36.1 mm carapace width); all others were <15 mm. Modal size of alpheids was 4.2 mm carapace length.

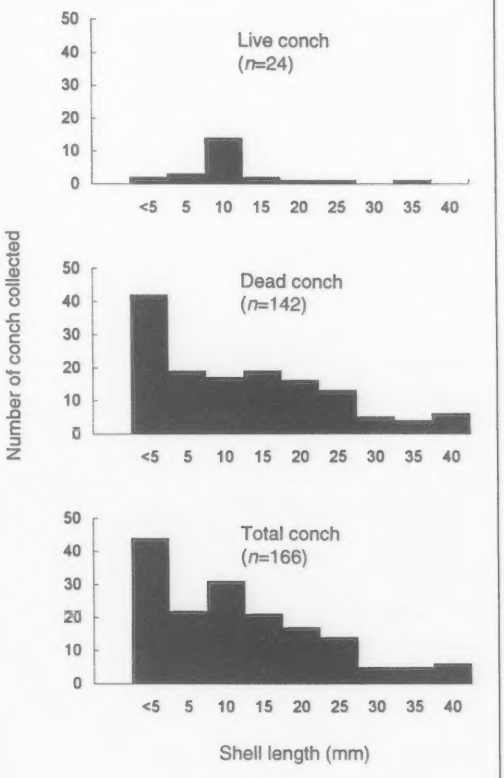


**Figure 3**

Length-frequency distribution for newly settled queen conch derived from all individuals, live and dead, collected from 11 dredge stations. Each value on the x axis represents a range of sizes starting with the lower end of the size interval (e.g. 5 represents 5.0–9.9 mm).

Live conch density had a high positive correlation with xanthid density ($r$=0.825, $P$=0.002), marginellid density ($r$=0.695, $P$=0.02), and total predator density ($r$=0.794, $P$=0.004), whereas relationships with densities of alpheids, portunids, and olivids were not significant ($P \geq 0.38$). There were no significant relationships with any of the predator families ($P \geq 0.15$) for dead or total conch densities.

## Discussion

### Age of postlarvae and settlement dates

The shell lengths of dredged newly settled conch provide insight into when settlement occurred. Using the middle of the week-long sampling period as the endpoint (28 August 1992), we estimated a summer average growth rate of 0.39 mm/day (Ray and Stoner, 1995), and considering settlement at 1.2 mm shell length (Davis, 1994), we determined that animals in the 10–15 mm modal size class would have settled 3–4 weeks earlier, between 24 July and 5 August. The largest live individual (38.5 mm) would have settled on about 24 May, and the smallest (3.3 mm) would have settled on 23 August. Dead conch ranged from 1.5 to 44 mm SL. According to the same assumptions, they would have been in the benthos for 1–110 days at the time of death. Therefore, settlement recorded in our samples began in early May and continued through at least late August. It should be pointed out that our intent was to collect young conch as close to the peak settlement period as possible, near the end of August. However, it is known that larvae are present in the water column near Lee Stocking Island until at least late September (Stoner and Davis, 1997), and our collections probably do not represent total settlement at Shark Rock in 1992.

**Table 5**

Size range and mode for three crustacean and two molluscan families of potential predators of newly settled conch collected at 11 dredge stations.

| Family | Measurement | Range (mm) | Mode (mm) |
|---|---|---|---|
| Xanthidae | carapace width | 1.0–14.5 | 1.5 |
| Alpheidae | carapace length | 1.0–7.8 | 4.2 |
| Portunidae | carapace width | 1.5–36.1 | 3.4 |
| Marginellidae | shell length | 1.7–6.1 | 3.2 |
| Olividae | shell length | 2.0–11.6 | 3.2 |

There are three possible explanations for the fact that dredging yielded relatively few live conch outside the 10–14.9 mm modal class. First, there could have been a major settlement event between late-July and early August. Queen conch larvae were collected at the Shark Rock nursery ground on 13 dates between late May and mid-September 1992 (Stoner and Davis, 1997); however, relatively few late-stage larvae were found in these collections, and it is impossible with the available data to determine if the modal size of survivors resulted from a period of high settlement rate. Nevertheless, this is the most parsimonious explanation. Second, the high number of year-class 0 conch in the 10–14.9 mm range may also be related to high survivorship in conch settling between late July and early August, compared to conch settling at other times. However, the steep decline in numbers of individuals with size could be a reflection of the natural, high mortality of small conch (dis-

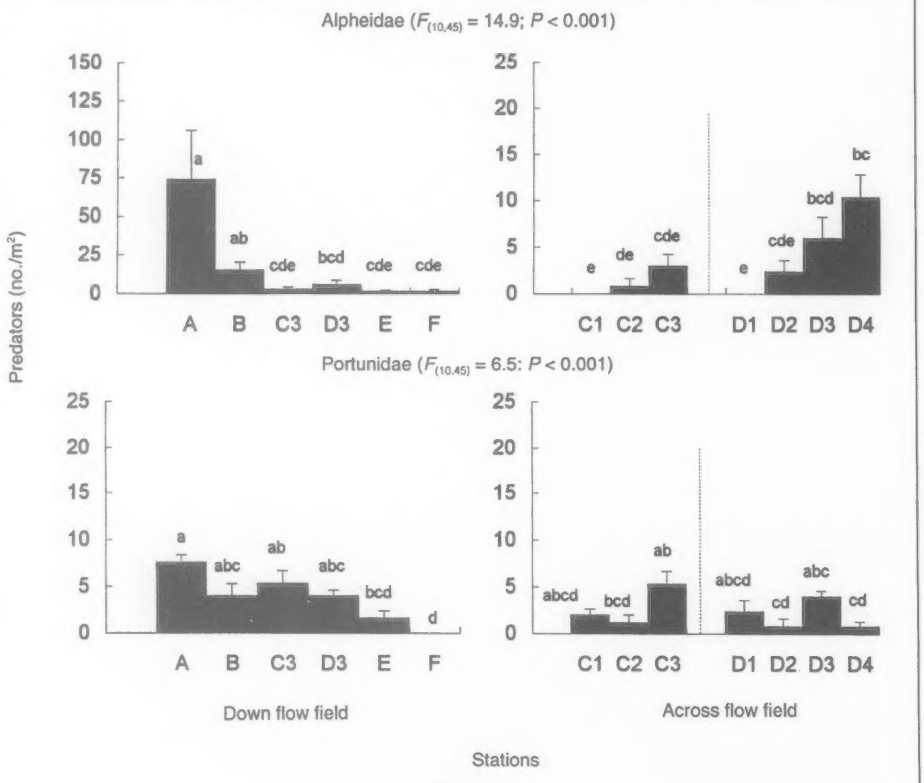


**Figure 4**

Density of three families of queen conch predators and total predators collected from 11 dredge stations located down and across the Shark Rock flow field. Total predators include alpheids, portunids, xanthids, as well as two mollusc families—olivids and marginellids (not shown individually). Stations C3 and D3 are illustrated in both dimensions for comparison. Values are mean ±SE ($n$=5 for all stations except C3, where $n$=6). Note different $y$ scale on each graph. $F$ and $P$ values are for 1-way ANOVAs made for each variable and flow-field dimension. Means that were not statistically different ($P$>0.05) are designated by similar letters (Tukey multiple comparison test on log-transformed data).

cussed below). This latter conclusion is supported by the fact that the modal size for dead conch was small (1–5 mm). Future studies should examine the interaction of settling date with food quality and quantity, growth, and predation rates. Few data of this type exist for sediment-dwelling invertebrates. Third, the smallest postlarval conch were probably undersampled. Despite the use of small mesh (1.2-mm), some of the smallest individuals may have passed through the dredge bags. Furthermore, shells of newly settled queen conch are frequently broken into fragments that would not be retained on a 1.2-mm mesh. Nevertheless, our study provides the first quantitative data on newly settled conch (<45 mm SL, <6 months postlarval age) and important insights into their early life history.

## Role of settlement and habitat on conch recruitment

In this study, large volumes of sediment had to be sorted to detect relatively low settlement densities (≤12/m$^2$) of queen conch over the traditional nursery. These densities were not surprising given that 1- and 2-year-old queen conch typically occur in aggregations with just 0.2–1.0 individual/m$^2$ (Stoner and Ray, 1993). The most intriguing findings are that settlement was concentrated in the traditional nursery ground and that recruitment to early benthic stages was directly correlated with settlement density over a large spatial scale. This is compatible with the prediction (Connell, 1985) that settlement will be most influential in predicting the recruitment of

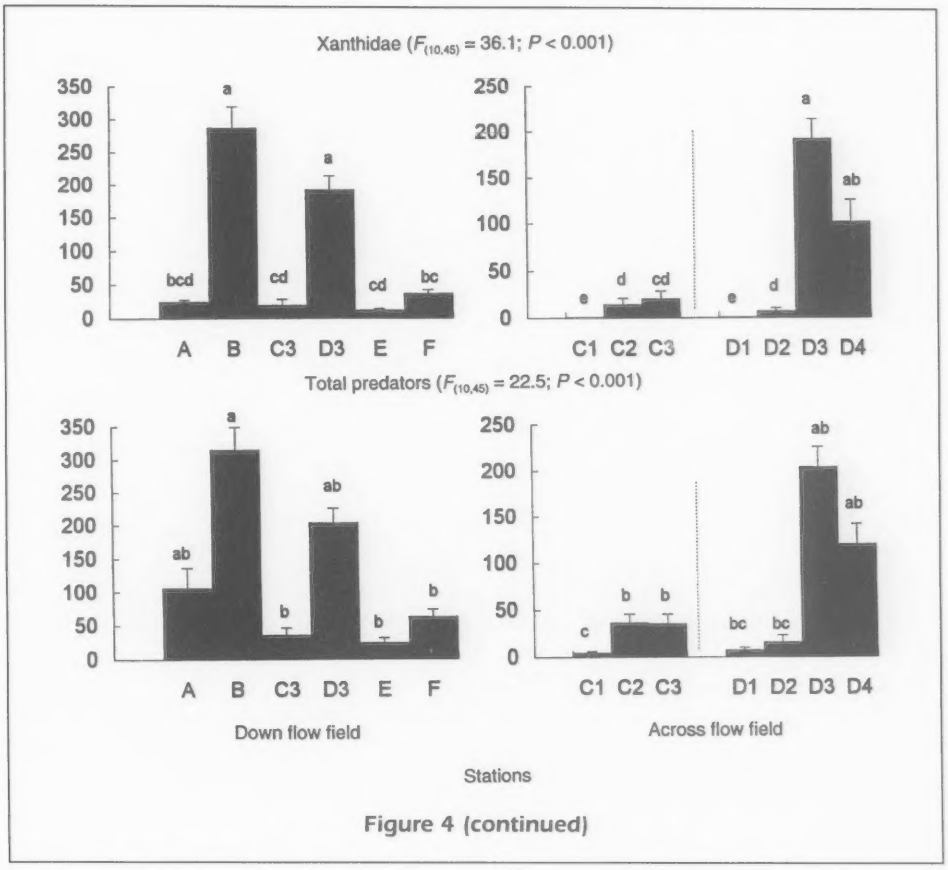
Xanthidae ($F_{(10,45)} = 36.1$; $P < 0.001$)
Total predators ($F_{(10,45)} = 22.5$; $P < 0.001$)
A B C3 D3 E F
C1 C2 C3 D1 D2 D3 D4
Down flow field
Across flow field
Stations

**Figure 4 (continued)**

benthic invertebrates when settlement occurs in low density

After an extensive review of the literature, Butman (1987) concluded that settlement patterns in invertebrates associated with soft sediments are a function of passive accumulation and deposition of larvae over spatial scales of kilometers and that active habitat selection occurs primarily over smaller scales (centimeters to meters). Data on the abundance of queen conch veligers over the Shark Rock tidal flow field (Stoner and Davis, 1997) may support this conclusion. Although newly hatched larvae (300–500 μm) were collected well beyond the Shark Rock nursery, at station F on 12 of 13 sampling dates in 1992, neither midstage larvae (500–900 μm), competent larvae (>900 μm), nor newly settled conch were ever collected there, suggesting that late-stage larvae are somehow concentrated at the nursery location. At maximum flood-tide current, velocity near the surface decreases by approximately 75% between station A, north of Lee Stocking Island, and Shark Rock. Near the bottom, the decrease in velocity is more than 95% (Stoner and Ray-Culp[1]). We also know that maximum flood tidal excursion in this flow field occurs near the Shark Rock nursery area on neap tide; therefore, the nursery is bathed in oligotrophic water (and perhaps larvae) from the Exuma Sound on every tide, whereas areas farther out on the bank are not (Jones, 1996; Stoner et al., 1996b). These hydrographic characteristics may result in the deposition of larvae in the long-term nursery area, or the lower botom-water velocities may allow them to settle.

Density of newly settled conch had a strong negative correlation with distance from the center of the traditional nursery ground but was relatively independent from all other environmental characteristics tested (i.e. depth and various qualities of sediments, seagrass, and macroalgae). This finding indicates that conch settlement cannot be explained

[1] Stoner, A. W., and M. Ray-Culp. 1997. Northeast Fisheries Science Center, Natl. Mar. Fish. Serv., NOAA, 74 Magruder Road, Highlands, NJ 07732. Unpubl. data.

simply by fine-scale hydrodynamic relationships associated with seagrasses and macroalgae, as observed for certain other invertebrates including mollusks (Eckman, 1987; Harvey et al., 1993, 1995). In fact, conch larvae settled in approximately equal densities across the seagrass gradient at transect D which spanned only 250 m. Therefore, the structure provided by macrophytes appears not to influence settlement of conch larvae even though older juveniles prefer seagrass habitats and are associated with an optimal shoot density (Stoner and Waite, 1990).

Although accumulation of competent larvae near the Shark Rock nursery is the most parsimonious explanation for the observed settlement and recruitment patterns, earlier experiments indicate that seemingly similar seagrass beds offer different qualities that have significant effects on larval and juvenile conch. Laboratory experiments have shown that macrophytes collected from stations within the Shark Rock nursery (B, C3, D3, D4) induced significantly higher metamorphosis than the same types of substrata collected outside the general nursery area (stations A, F) (Davis and Stoner, 1994). Growth rates of newly settled conch (1.2 mm) fed seagrass detritus from the different sources reflected metamorphic responses on the same substrata (Stoner et al., 1996a), and when 1-yr-old juvenile conch were transplanted to stations A and F, growth rates were low in comparison with those in the Shark Rock nursery (Stoner et al., 1994). It is clear, therefore, that the nursery area is trophically unique, despite visual similiarity to surrounding areas.

Micro-organism films that coat the sediment in soft-bottom communities are known to be important inducers of metamorphosis in conch and other invertebrates, most likely because they are associated with favorable nutritional requirements for postlarvae (Scheltema, 1961; Gray, 1974; Davis and Stoner, 1994; Stoner et al., 1996a). Characteristics that make the Shark Rock nursery an attractive location for settling larvae and an ecologically suitable habitat for juveniles probably stem from hydrodynamic properties of the location that affect nutrient cycling and productivity patterns in certain algal foods for conch (Stoner et al., 1994, 1996b). Field manipulations will be needed to distinguish direct effects of hydrodynamics (i.e. larval transport and retention) from indirect effects such as hydrographic mediation of biological productivity, habitat choices, and postsettlement processes.

Postlarval conch recruited to the same habitats traditionally occupied by 1- and 2-yr-old animals. This association is probably not the result of conspecific attraction because settlement was equally high at stations with and without older conspecifics. This result corroborates an earlier laboratory study showing that cues associated with previously settled juveniles (slime trails, feces, and the older conch themselves) did not elicit larval metamorphosis (Davis and Stoner, 1994).

### The role of micropredators on conch recruitment

Although settlement of queen conch was relatively independent of habitat features other than location, predator distributions were highly correlated with seagrass shoot density, detrital abundance, and sediment organics and grain size. Numerous studies have shown that animal abundances in seagrass beds are correlated with certain measures of habitat complexity such as seagrass blade density or biomass, detrital biomass, leaf characteristics, or rhizome structure (Orth et al., 1984; Stoner and Lewis, 1985). The association between benthic macrofauna and physical structure may be related to food abundance or predation, or both. There is abundant experimental evidence that the physical structure provided in seagrass beds reduces predation rates on invertebrates (Heck and Wilson, 1987; Heck and Crowder, 1991). Potential conch predators dredged in this study were prey species themselves, and they undoubtedly derived some measure of protection or nutrition from the habitat, or both.

Predation on early postsettlement stages can be an important process affecting the number of invertebrate settlers that survive to recruit into a population (Thorson, 1966; Keough and Downes, 1982; Osman and Whitlach, 1995). Heavy losses to micropredators during the first days or weeks after settlement can severely diminish or eliminate a prey species, and even regulate community composition (Osman et al., 1992; Osman and Whitlatch, 1995). It is apparent from the length frequency of dead conch collected in this study that very high mortality occurs immediately after settlement, when conch are <5-mm shell length. Queen conch have many predators at this size (Ray-Culp et al., 1997), and one of the most important is probably the xanthid crab *Micropanope* sp., which was the most abundant invertebrate counted in dredge samples. The crab is capable of killing conch that are up to 0.5 times its own carapace width (Ray-Culp et al.[2]). Although a large proportion of the xanthids collected were too small (mode=1.5 mm) to kill even newly settled conch

[2] Ray-Culp, M., M. Davis, and A. W. Stoner. 1998. Escaping the xanthid crab gauntlet—the role of size, density and habitat for newly-settled queen conch. Caribbean Marine Research Center, 805 E. 46th Place, Vero Beach, FL 32963. Unpubl. manuscr.

(1.2 mm), xanthids up to 10 mm were far more abundant than conch, and they undoubtedly play a major role in conch mortality. The high positive correlation between live conch and xanthids suggests that conch settle in areas prone to high predator abundance. However, with typical summer growth rates, mentioned earlier, conch would escape predation by xanthids in about 10 days when they reach 5 mm shell length. Consequently, predator-prey relationships associated with the early postsettlement stages may be highly dynamic, with suites of predators shifting rapidly over time.

There was an increase in conch survivorship from bare sand to moderate density seagrass over both cross-channel transects. A similar trend was obtained experimentally when year-class 0 and 1 juveniles were tethered over an analogous seagrass gradient near transect D (Ray and Stoner, 1994, 1995). Highest mortality occurred on bare sand and in highest seagrass biomass whereas lowest mortality occurred in moderate biomass. It appears that a certain amount of seagrass structure provides protection, but too much is detrimental. However, in the down flow field dimension, survivorship was highest within the traditional nursery ground, and distribution is clearly related to both settlement and survivorship in this dimension.

### Identifying critical habitats

Our findings have important management implications for queen conch and other species associated with seagrass beds. First, it may not be possible to determine the value of a particular site for a species on the basis of simple habitat maps. Even descriptions of the beds that include seagrass species composition, biomass, and shoot density do not provide adequate information to identify critical habitats for conch. Value of specific seagrass bed locations also depends upon hydrography, larval retention, larval settlement, predator abundance, and the related survivorship. Persistence in the locations of queen conch nurseries near Lee Stocking Island (Stoner et al., 1996b; Jones, 1996) indicates that these factors are relatively constant over periods of several years, and that the specific locations may be as "critical" as habitat type. Only certain seagrass beds are suitable for queen conch; these must be identified and protected. Furthermore, in many areas of the Caribbean, conch populations have been devastated by overfishing, and there is an intense interest in rehabilitating them through releases of hatchery-reared juveniles. Transplant experiments have shown that juvenile conch will survive and grow only in very specific locations, and releases must be made in locations that ensure economically acceptable survivorship (Stoner, 1994). Clearly, thorough knowledge of distributional mechanisms is necessary to make predictions on the habitat requirements of queen conch and other managed species.

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## Literature cited

**Appeldoorn, R. S.**
**1988.** Age determination, growth, mortality and age of first reproduction in adult queen conch, *Strombus gigas* L., off Puerto Rico. Fish. Res. 6:363–378.
**1994.** Queen conch management and research: status, needs and priorities. *In* R. S. Appeldoorn and B. Rodriguez (eds.), Queen conch biology, fisheries and mariculture, p. 301–319. Fundación Científica Los Roques, Caracas, Venezuela.

**Brook, I. M.**
**1979.** A portable suction dredge for quantitative sampling in difficult substrates. Estuaries 2:54–58.

**Butman, C. A.**
**1987.** Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. Oceanogr. Mar. Biol. Ann. Rev. 25:113–165.

**Butman, C. A., and J. P. Grassle.**
**1992.** Active habitat selection by *Capitella* sp. I larvae. I. Two-choice experiments in still water and flume flows. J. Mar. Res. 50:669–715.

**Connell, J. H.**
**1985.** The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. J. Exp. Mar. Biol. Ecol. 93:11–45.

**Davis, M.**
**1992.** Predation of hatchery-reared juvenile queen conch, *Strombus gigas* (L.) by juvenile spiny lobsters, *Panulirus argus* (L.). M.S. thesis, Florida Institute of Technology, Melbourne, FL, 37 p.
**1994.** Short-term competence in larvae of queen conch *Strombus gigas*: shifts in behavior, morphology and metamorphic response. Mar. Ecol. Prog. Ser. 104:101–108.

**Davis, M., C. A. Bolton, and A. W. Stoner.**
**1993.** A comparison of larval development, growth, and shell morphology in three Caribbean *Strombus* species. Veliger 36:236–244.

**Davis, M., and A. W. Stoner.**
**1994.** Trophic cues induce metamorphosis of queen conch larvae (*Strombus gigas* Linnaeus). J. Exp. Mar. Biol. Ecol. 180:83–102.

**Day, R. W., and G. P. Quinn.**
**1989.** Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59:433–463.

**Eckman, J. E.**
**1987.** The role of hydrodynamics in recruitment, growth, and survival of *Argopecten irradians* (L.) and *Anomia simplex* (D'Orbigny) within eelgrass meadows. J. Exp. Mar. Biol. Ecol. 106:165–191.

**Folk, R. L.**
**1966.** A review of grain-size parameters. Sedimentology 6:73–93.

**Grassle, J. P., C. A. Butman, and S. W. Mills.**
**1992.** Active habitat selection by *Capitella* sp. I larvae. II. Multiple-choice experiments in still water and flume flows. J. Mar. Res. 50:717–743.

**Gray, J. S.**
**1974.** Animal-sediment relationships. Oceanogr. Mar. Biol. Ann. Rev. 12:223–261.

**Hadfield, M. G., and D. Scheuer.**
**1985.** Evidence for a soluble metamorphic inducer in *Phestilla*: ecological, chemical and biological data. Bull. Mar. Sci. 37:556–566.

**Harvey, M., E. Bourget, and R. G. Ingram.**
**1995.** Experimental evidence of passive accumulation of marine bivalve larvae on filamentous epibenthic structures. Limnol. Oceanogr. 40:94–104.

**Harvey, M., E. Bourget, and G. Miron.**
**1993.** Settlement of Iceland scallop *Chlamys islandica* spat in response to hydroids and filamentous red algae: field observations and laboratory experiments. Mar. Ecol. Prog. Ser. 99:283–292.

**Heck, K. L., Jr., and L. B. Crowder.**
**1991.** Habitat structure and predator-prey interactions in vegetated aquatic systems. *In* S. S. Bell, E. D. McCoy, and H. R. Mushinsky (eds.), Habitat structure: the physical arrangement of objects in space, p. 281–299. Chapman and Hall, New York, NY.

**Heck, K. L., Jr., and K. A. Wilson.**
**1987.** Predation rates on decapod crustaceans in latitudinally separated seagrass communities: a study of spatial and temporal variation using tethering techniques. J. Exp. Mar. Biol. Ecol. 107:87–100.

**Iversen, E. S., and D. E. Jory.**
**1997.** Mariculture and enhancement of wild populations of queen conch (*Strombus gigas*) in the western Atlantic. Bull. Mar. Sci. 60:929–941.

**Iversen, E. S., S. P. Bannerot, and D. E. Jory.**
**1989.** Evidence of survival value related to burying behavior in queen conch *Strombus gigas*. Fish. Bull. 88:383–387.

**Iversen, E. S., D. E. Jory, and S. P. Bannerot.**
**1986.** Predation on queen conchs, *Strombus gigas*, in the Bahamas. Bull. Mar. Sci. 39:61–75.

**Iversen, E. S., D. E. Jory, and D. J. DiResta.**
**1994.** Research on the first year queen conch, *Strombus gigas*, relevant to fisheries management. Proc. Gulf Caribb. Fish. Inst. 43:498–505.

**Iversen, E. S., E. S. Rutherford, S. P. Bannerot, and D. E. Jory.**
**1987.** Biological data on Berry Islands (Bahamas) queen conchs, *Strombus gigas*, with mariculture and fisheries management implications. Fish. Bull. 85:299–310.

**Jones, R. L.**
**1996.** Spatial analysis of biological and physical features associated with the distribution of queen conch, *Strombus gigas*, nursery habitats. M.S. thesis, Florida Institute of Technology, Melbourne, FL, 98 p.

**Jory, D. E.**
**1982.** Predation by tulip snails, *Fasciolaria tulipa*, on queen conchs, *Strombus gigas*. M.S. thesis, Univ. of Miami, FL, 73 p.

**Keough, M. J., and B. J. Downes.**
**1982.** Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia 54:348–352.

**Luckenbach, M. W.**
**1984.** Settlement and early post-settlement survival in the recruitment of *Mulinia lateralis* (Bivalvia). Mar. Ecol. Prog. Ser. 17:245–250.

**Marshall, L. S.**
**1992.** Survival of juvenile queen conch, *Strombus gigas*, in natural habitats: impact of prey, predator and habitat features. Ph.D. diss., College of William and Mary, Williamsburg, VA, 144 p.

**McBride, E. F.**
**1971.** Mathematical treatment of size distribution data. *In* R. E. Carver (ed.), Procedures in sedimentary petrology, p. 9–27. Wiley, New York, NY.

**Orth, R. J., K. L. Heck Jr., and J. van Montfrans.**
**1984.** Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350.

**Osman, R. W., and R. B. Whitlatch.**
**1995.** Predation on early ontogenetic life stages and its effect on recruitment into a marine epifaunal community. Mar. Ecol. Prog. Ser. 117:111–126.

**Osman, R. W., R. B. Whitlatch, and R. J. Malatesta.**
**1992.** Potential role of micro-predators in determining recruitment into a marine community. Mar. Ecol. Prog. Ser. 83:35–43.

**Randall, J. E.**
**1964.** Contributions to the biology of the queen conch, *Strombus gigas*. Bull. Mar. Sci. Gulf. Caribb. 14:246–295.

**Ray-Culp, M., M. Davis, and A. W. Stoner.**
**1997.** The micropredators of settling and newly-settled queen conch (*Strombus gigas* Linnaeus). J. Shellfish Res. 16:423–428.

**Ray, M., and A. W. Stoner.**
**1994.** Experimental analysis of growth and survivorship in a marine gastropod aggregation: balancing growth with safety in numbers. Mar. Ecol. Prog. Ser. 105:47–59.
**1995.** Growth, survivorship, and habitat choice in a newly settled seagrass gastropod, *Strombus gigas*. Mar. Ecol. Prog. Ser. 123:83–94.

**Sandt, V. J., and A. W. Stoner.**
**1993.** Ontogenetic shift in habitat by early juvenile queen conch, *Strombus gigas*: patterns and potential mechanisms. Fish. Bull. 91:516–525.

**Scheltema, R. S.**
**1961.** Metamorphosis of the veliger larva of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. Biol. Bull. 120:92–109.

**Stoner, A. W.**
**1994.** Significance of habitat and stock pre-testing for enhancement of natural fisheries: experimental analyses with queen conch *Strombus gigas*. J. World Aquacult. Soc. 25:155–165.

**Stoner, A. W., and M. Davis.**
**1997.** Abundance and distribution of queen conch veligers (*Strombus gigas* Linne) in the Central Bahamas: I. Horizontal patterns in relation to reproductive and nursery grounds. J. Shellfish. Res. 16:7–18.

**Stoner, A. W., M. D. Hanisak, N. P. Smith, and R. A. Armstrong.**
**1994.** Large-scale distribution of queen conch nursery habi-

tats: implications for stock enhancement. *In* R. S. Appeldoorn and B. Rodriguez (eds.), Queen conch biology, fisheries and mariculture, p. 169–189. Fundación Científica Los Roques, Caracas, Venezuela.

**Stoner, A. W., and F. G. Lewis III.**
**1985.** The influence of quantitative and qualitative aspects of habitat complexity in tropical sea-grass meadows. J. Exp. Mar. Biol. Ecol. 94:19–40.

**Stoner, A. W., P. A. Pitts, and R. A. Armstrong.**
**1996b.** The interaction of physical and biological factors in the large-scale distribution of queen conch populations in seagrass meadows. Bull. Mar. Sci. 58:217–233.

**Stoner, A. W., and M. Ray.**
**1993.** Aggregation dynamics in juvenile queen conch (*Strombus gigas*): population structure, mortality, growth, and migration. Mar. Biol. 116:571–582.

**Stoner, A. W., M. Ray, R. A. Glazer, and K. J. McCarthy.**
**1996a.** Metamorphic responses to natural substrata in a gastropod larva: decisions related to postlarval growth and habitat preference. J. Exp. Mar. Biol. Ecol. 205:229–243.

**Stoner, A. W., and V. J. Sandt.**
**1992.** Population structure, seasonal movements and feeding of queen conch, *Strombus gigas*, in deep-water habitats of the Bahamas. Bull. Mar. Sci. 51:287–300.

**Stoner, A. W., and J. M. Waite.**
**1990.** Distribution and behavior of queen conch, *Strombus gigas*, relative to seagrass standing crop. Fish. Bull. 88:573–585.

**Thorson, G.**
**1966.** Some factors influencing the recruitment and establishment of marine benthic communities. Neth. J. Sea Res. 3:267–293.

**Vermeij, G. J.**
**1982.** Gastropod shell form, breakage, and repair in relation to predation by the crab *Calappa*. Malacologia 23:1–12.
**1987.** Evolution and escalation: an ecological history of life. Princeton Univ. Press, Princeton, NJ, 527 p.

# Growth trajectory of the larval Japanese sardine, *Sardinops melanostictus*, transported into the Pacific coastal waters off central Japan

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**Abstract.**—Late larvae (15–30 mm TL) of the Japanese sardine, *Sardinops melanostictus*, are commercially exploited in fishing grounds along the Pacific coast of western and central Japan. Concentrated shoals of late larvae in the shallow (15–30 m deep) coastal (4–6 miles from the coast) fishing grounds enable fishermen to catch as much as several hundred metric tons (t) (several billion larvae in number) per month. Growth trajectories of sardine larvae caught in the fishing ground off Atsumi Peninsula in central Japan were individually backcalculated by using the biological intercept method based on the allometric relationship between otolith radius and fish length. Growth rates for larvae up to 13–21 d were high, ranging from 0.79 to 0.85 mm/d, but declined after reaching size of immigration (13–19 mm TL) from the offshore waters to the coastal fishing grounds. The decline of growth rate in the late larval stage seemed to be related to the concentration of late larvae in the fishing grounds, the result of onshore intrusions of offshore Kuroshio waters. Total lengths at age 20 d were significantly smaller in 1990 (total catch of larval sardine was 720 t) than in 1991 (total catch 300 t) in spite of a higher sea surface temperature in 1990 in the coastal habitat. This may have resulted from a larger population of late larvae on the fishing ground in 1990 than in 1991.

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Fish. Bull. 96:900–907 (1998).

Late larvae of the Japanese sardine *Sardinops melanostictus* and the Japanese anchovy, *Engraulis japonicus*, termed *shirasu* in Japan, are fished by boat seiners in waters along the Pacific coast of western and central Japan. The coastal waters (4–6 miles from the coast) off Atsumi Peninsula (Fig. 1) are one of the major fishing grounds for *shirasu*. Depth of the sea bed of the fishing grounds is about 20 m, with a range from 15 to 30 m. The *shirasu* fishery for sardine larvae begins in March and continues to December, with the major effort shifting to anchovy in early summer. The annual catch of sardine larvae in the waters ranged from 700 to 2000 metric tons (t), 7–20 billion larvae, 1980–88. However, between 1989 and 1991 the catch declined to 300–700 t (Fig. 2). Since 1992, catches have been as low as several tens of tons, with an exception of about 400 t in 1993.

Growth rates of fish larvae affect their survival and recruitment to the adult population (Anderson, 1988). From life-stage table analyses, growth rate has been shown to be an important determinant of year-class strength by delimiting the duration of a particular life stage with high instantaneous mortality (Lo et al., 1995; Butler et al., 1996). Watanabe et al. (1995) showed that recruitment failures of the Japanese sardine in 1988–91 could not be explained by mass mortality at the first feeding stage. Cumulative mortality after the first feeding stage of the sardine, which may be a function of growth rate, is likely to have determined the recruitment in these years. Meekan and Fortier (1996) examined early life growth and survival of the Atlantic cod, *Gadus morhua*, and showed that fast-growing pelagic larvae survived better through the larval stage and dominated in the cohort of demersal juveniles. Campana (1996) found a positive correlation between growth rates in juvenile Atlantic cod and subsequent year-class strengths, which enabled him to predict recruitment from the juvenile growth rates.

Available population size of late larval sardine in the *shirasu* fishing grounds could be affected by larval growth. The size range of larvae caught in the *shirasu* fishery is usually from 15 to 30 mm TL. Because the duration time of this size

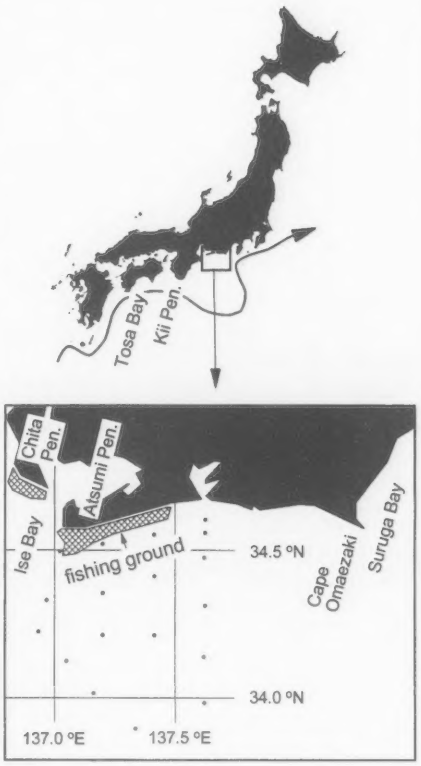


**Figure 1**

Location of *shirasu* fishing grounds in the coastal waters along Atsumi Peninsula. Minor fishing grounds are found in Ise Bay at the tip of Chita peninsula. Dots indicate locations of monthly SST observations. A solid line along the Pacific coast of western and central Japan denotes an example of the meandering Kuroshio current axis.

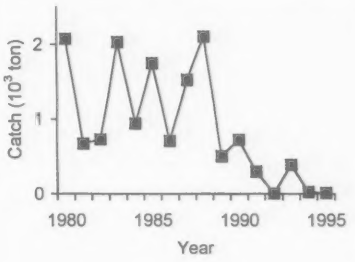


**Figure 2**

Annual catch of sardine *shirasu* in the coastal waters of Atsumi and Chita Peninsulas after 1980.

range is a function of growth rate, slower growth results in longer duration time and an increase in the available number of larvae. Watanabe and Kuroki (1997) backcalculated the growth history of larval sardine *Sardinops melanostictus* in the coastal waters off Miyazaki in western Japan. They found that growth rates reached a maximum (0.80–0.85 mm/d) at around 10–12 mm in total length (TL) but slowed thereafter, exhibiting asymptotic growth trajectories. According to this growth pattern, duration time from 15 to 30 mm TL was calculated to be 35 days. If, however, growth rates of 0.80–0.85 mm/d were maintained in the late larval stage, duration time of the same size range could be as short as 18 days.

Growth rate in early life stages is thus important as a potential determinant of year-class strength as well as of available stock sizes of *shirasu* larvae of the Japanese sardine. The daily growth rate of fish can be estimated by regressing size-at-age data to a growth model (Campana and Jones, 1992). When we apply this method, we need data points throughout an age range, from first feeding up to the fish size of concern (Watanabe et al., 1997). Because the size range of sardine larvae fished by the boat seine fishery is usually from 15 to 30 mm, we do not have a complete range of data points for early larvae, and therefore the regression method is not applicable to describe the growth history from first feeding to size at capture. Instead, backcalculation of size at age from the relationship between otolith radius and fish length makes it possible to draw a growth trajectory for individual fish (Campana, 1990; Campana and Jones, 1992). We backcalculated growth trajectories of individual larval sardines caught in coastal waters off Atsumi Peninsula, 1990 and 1991, and compared them with growth trajectories of larvae from other waters.

## Materials and methods

### Larval sampling

Two *shirasu* fishing boats, towing a seine net, were used to sample waters off Atsumi Peninsula and in Ise Bay (Fig. 1). They usually departed before dawn from the fishing port, together with a catch-loading boat, set the net several times in the morning, and returned to the port for offloading. Catches were stored with ice before landing. In our study, we randomly sampled *shirasu* larvae from catches in the ports of Morozaki and Toyohama (tip of Chita Peninsula) four times in 1990 (11, 16, 27 April and 7 May) and four times in 1991 (15, 23 April and 7, 14 May).

The larvae were classified as Japanese sardine, *S. melanostictus*, Japanese anchovy, *E. japonicus*, and round herring, *Etrumeus teres*. Sardine larvae were preserved in 80% ethanol for otolith examinations.

### Otolith measurement

Total lengths (TL) of sardine larvae were measured to the nearest 0.1 mm with an optical comparator. Sagittal otoliths were dissected and cleaned under a binocular microscope, mounted on a glass plate with enamel resin, and used for measurements and counts of daily growth rings. We used the otolith measurement system (Ratoc System Engineering Inc.) composed of a light microscope, a video camera and monitor, and an image analyzer controlled by computer. Because the otolith had not yet developed a rostrum and because we were not able to determine its orientation at the larval stage, we measured radii of all daily rings along the maximum radius of the otolith.

### Growth backcalculation

The relationship between larval TL and maximum otolith radius (OR) was considered for larvae sampled. Plots of TL against OR can be expressed by an allometric relationship (see "Results" section). An allometric OR-TL relationship has previously been demonstrated in larval *S. melanostictus* from different waters (Watanabe and Kuroki, 1997). Otolith growth rings have been found to be deposited on a daily basis in *S. melanostictus*, with the first ring being formed on the third day of hatching (the day of first feeding) when larvae are reared at 18°C (Hayashi et al., 1989). The relationship of the $i$th otolith ring radius ($OR_i$) and $TL$ on the day of the $i$th ring formation ($TL_i$) is considered to be expressed by an allometric formula, $TL_i = a \times OR_i^b$, for individual larvae. We determined the allometric parameters $a$ and $b$ for each larva by using the biological intercept method (Campana, 1990; Campana and Jones, 1992) and a size of first feeding larvae (first otolith daily ring deposition) of 5.0 mm TL (Watanabe and Kuroki, 1997). The solution of the following two equations gives us $a$ and $b$ for each larva:

$$TL_1 = a \times OR_1^b \text{ and } TL_{capture} = a \times OR_{capture}^b,$$

where $TL_1$ = total length (mm) at the first ring deposition which was fixed at 5.0 mm;
$OR_1$ = the measured radius of the first daily ring;
$TL_{capture}$ = the measured total length (μm) at capture; and
$OR_{capture}$ = otolith radius (μm) at capture.

$TL_i$ of each larva was thus calculated from the formula for each larva independently. Mean ±SD (standard deviation) of TL at ages from 4 d up to a certain age, with at least 10 backcalculated TLs, was calculated for the March- and April-hatched cohorts for the two years of study. Differences in mean TLs at ages 10, 15, 20, and 25 d were examined between the same month of hatch cohorts in 1990 and 1991 by using Student's $t$-test, when variances were equal, or by Welch's $t$-test when they were not equal.

### Sea surface temperature distribution

Sea surface temperatures (SST) in and around the *shirasu* fishing ground were measured monthly at fixed stations off Atsumi Peninsula (Fig. 1). We used data from April and May, 1990 and 1991, to describe temperature distributions in coastal waters.

## Results

The SST in coastal waters off Atsumi Peninsula was in the range of 16–19°C during 23–25 April and 8–9 May 1990. From 23 to 25 April, the isotherms ran nearly parallel to the coast line but from 8 to 9 May offshore waters warmer than 18°C intruded into the coastal fishing ground (Fig. 3). In 1991, the SST during 2–3 April and 8–9 May ranged from 11 to 18°C. Cold waters extruded from Ise Bay covered part of the coastal fishing ground. The SST in the fishing grounds were 1.5–2.5°C lower in 8–9 May 1991 than in the corresponding season in 1990.

Local government permits the *shirasu* fishery to operate year round in these fishing grounds, but catches were zero in January–February 1990 and January–March 1991 (Fig. 4). Japanese sardine larvae were caught mainly in April in these years. The major target species of the *shirasu* fishery shifted to Japanese anchovy after May. A monthly catch of sardine larvae was 430 t in April 1990, declining to 175 t in April 1991. Annual catches of sardine larvae were 724 t and 298 t in 1990 and 1991, respectively.

Total length of larval sardines caught in the *shirasu* fishery were 15–27 mm in mid-April 1990 (Fig. 5). Frequency of larvae smaller than 15 mm TL increased after late April in this year, with sizes of 11–20 mm. In 1991, the modal size and range of the larvae remained relatively constant at 22–25 and 20–27 mm TL, respectively, mid-April to mid-May (Fig. 5).

Sardine larvae fished on 11 and 16 April 1990 were aged from 14 to 39 d after hatching. All of them hatched in March (Fig. 6). Larvae caught on 27 April

and 7 May were young, from 10 to 23 d. Except for one larva hatched on 31 March (not used for backcalculation), all larvae caught on 27 April and 7 May hatched in April 1990. In 1991, ages of sardine larvae were 19–30 d. Larvae caught on 15 April were all hatched in March. Those caught on 23 April were composed of March- and April-hatched fish. All larvae caught on 5 and 14 May were hatched in April except one which was hatched on 24 March (not used for backcalculation).

Plots of TL (mm) on OR (μm) of all individuals could be expressed in an allometric formula (Fig. 7). Size at age of individual larva was backcalculated on the basis of allometric OR-TL relationship for each larva from first feeding to capture. The 1990 March-hatched cohort grew linearly to 25 d when fish reached 20.5 mm TL (Fig. 8). Mean growth rate of the cohort from 4 d (6.3 mm TL) to 25 d was 0.68 mm/d. Growth in the April-hatched cohort in 1990 was linear to about 13 d (13.1 mm TL, 0.83 mm/d), then slowed down. In the 1991 March- and April-hatched cohorts, growth was linear to 21 d (21.2 mm TL, 0.85 mm/d), and 20 d (19.2 mm TL, 0.79 mm/d), respectively, declining thereafter. Larval TL at which growth started to decline occurred at the approximate size when immigration into the coastal fishing grounds occurred in both months.

Total lengths at 15 d in the 1990 March- and April-hatched cohorts were 14.2 ±1.2 and 14.9 ±1.4 mm, respectively, whereas in 1991 the March-hatched cohort reached 16.3 ±1.5, and the April cohort 15.8 ±1.3 mm. Backcalculated TLs of March- and April-hatched cohorts were significantly smaller in 1990 than in corresponding hatching month in 1991 at 15, 20, and 25 d (Table 1).

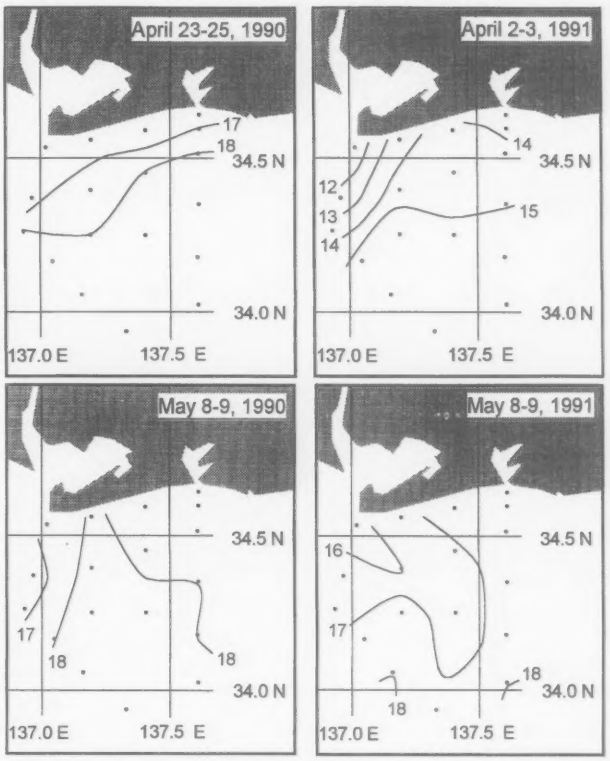


**Figure 3**

Sea surface temperature distribution off Atsumi Peninsula in April and May of 1990 and 1991.

## Discussion

Larvae of *S. melanostictus* and *E. japonicus* have been reported to be transported from the offshore Kuroshio area to the coastal fishing grounds along the Pacific coast in central Japan by onshore intrusions of Kuroshio waters (Tsuji, 1983; Muranaka, 1984; Mitani, 1990). This is the case in the coastal fishing grounds off Atsumi Peninsula, because great densities of *S. melanostictus* eggs were detected in the offshore waters along the Kuroshio Current in 1990 and 1991 (Ishida and Kikuchi, 1992; Zenitani et al., 1995; Watanabe et al., 1996). Onshore intrusions of Kuroshio waters often develop when the Kuroshio meanders in the waters off central Japan (Kobayashi et al., 1986; Kasai, 1995). The available population size of *S. melanostictus* larvae in coastal fishing grounds off Atsumi Peninsula and adjacent waters is a positive function of the latitudinal distance from Cape Omaezaki to the Kuroshio axis (Fig. 1) (Kishida et al., 1994). This distance in April and May 1991 measured approximately 125 nautical miles (n mi) (Maritime Safety Agency, 1991), which was less than that in 1990 (200 n mi) (Maritime Safety Agency, 1990). Oceanic conditions were less favorable for onshore larval transport in 1991 than in 1990. As seen in Figure 3, SSTs in coastal waters were lower in 1991 than in 1990, a feature that was indicative of limited intrusion of the warm Kuroshio waters to the coastal area. Annual catch of sardine *shirasu* in 1991 in the waters off Atsumi Peninsula (including small catches in Ise Bay) decreased to 41%

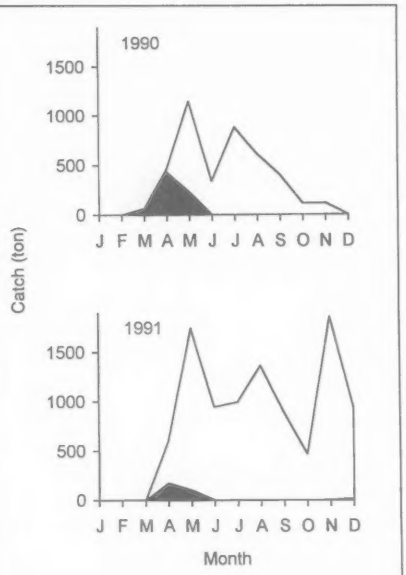


**Figure 4**

Monthly catch of late larvae of sardine (closed) and anchovy (open) by the *shirasu* fishery in the coastal waters off Atsumi and Chita Peninsulas.

of the 1990 catch. This was due partly to unfavorable oceanic conditions.

Growth rates of sardine larvae estimated in this study were 0.79–0.85 mm/d to a size when they immigrated to the fishing grounds as 1990 April- and 1991 March- and April-hatched cohorts, but later declined to 0.6–0.7 mm/d (Fig. 8). This decline in growth was similar to the asymptotic growth of larval *S. melanostictus* in the *shirasu* fishing grounds in western Japan (Watanabe and Kuroki, 1997). Fishermen for *shirasu* locate a concentrated larval shoal by echo sounder (Mitani, 1987) and catch large numbers of larvae in relatively narrow fishing grounds (Fig. 1). In mid-April 1990, 100 t (ca. one billion in number) per day of sardine larvae were caught in the fishing ground in our study.[1] Turbidity is a factor that helps to retain concentrated larval shoals in the coastal fishing grounds (Funakoshi, 1988). Uotani et al. (1993) demonstrated experimentally that *E. japonicus* larvae showed a strong positive taxis to turbidity and tended to stay in the turbid water. Reduction of larval growth, after reaching the size when immigration to the *shirasu* fishing grounds occurred, is likely to be related to concentrations of large numbers of larval sardines in the narrow fishing grounds as a result of intrusions of offshore waters (Muranaka, 1984).

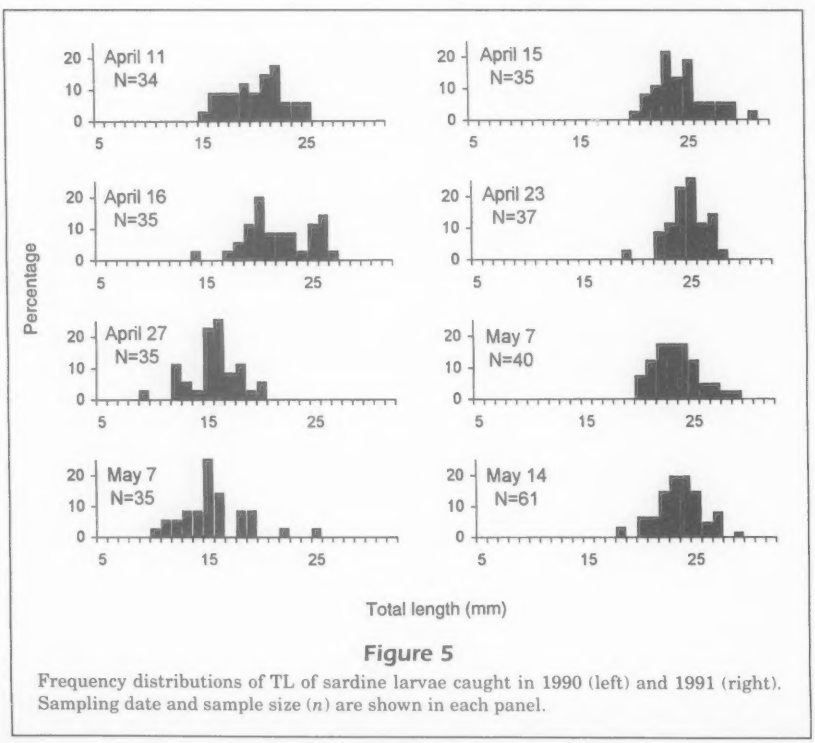


**Figure 5**

Frequency distributions of TL of sardine larvae caught in 1990 (left) and 1991 (right). Sampling date and sample size ($n$) are shown in each panel.

The modal size of sardine larvae caught on April 27 and May 7 (April-hatched cohort) in 1990 was exceptionally smaller (15–16 mm TL) than those in the other larval groups (Fig. 5). The mesh aperture of the net of the *shirasu* fishery is 2.1 mm at the codend. The diagonal of the mesh is 3.0 mm, which is much larger than the body depth of 15–16 mm sardine larvae (about 1.3 mm). A large proportion of larvae smaller than 15 mm could, therefore, be extruded from the mesh at the codend (Smith and Richardson, 1970). Nevertheless, many larvae smaller than 15 mm

[1] 1990. Marine Resources Research Center of Aichi Fisheries Research Institute, 2-1 Toyoura, Toyohama, Minami-Chita, Chita-gun, Aichi 47034, Japan. Unpubl. data

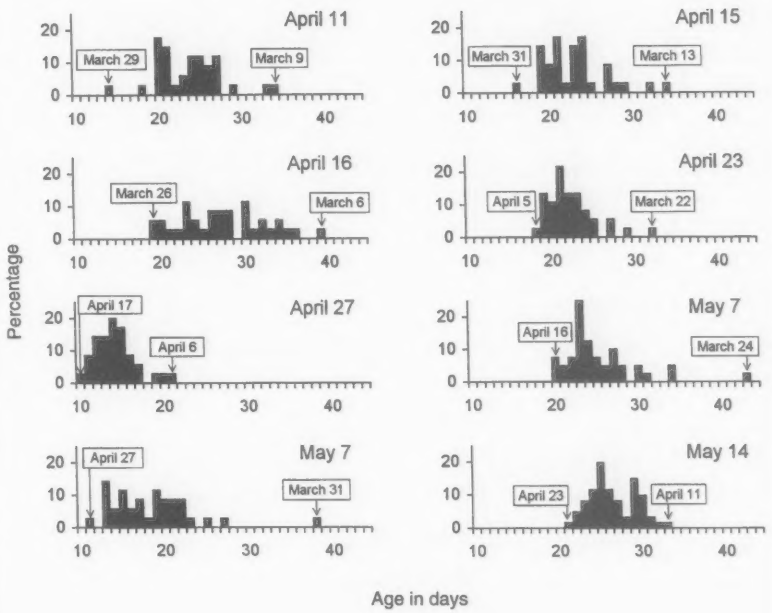


**Figure 6**

Frequency distribution of age of sardine larvae caught on different dates in 1990 (left) and 1991 (right). Boxes show the earliest or latest hatch date of the group.

**Table 1**

Back-calculated TLs (in mm) of sardine larvae at ages 10, 15, 20, and 25 d after hatching.

| | | 10 | | | 15 | | | 20 | | | 25 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hatching month | | Mean | SD | *n* | Mean | SD | *n* | Mean | SD | *n* | Mean | SD | *n* |
| 1990 | March | 10.8** | 0.9 | 69 | 14.1** | 1.2 | 68 | 17.5** | 1.4 | 65 | 20.5** | 1.3 | 35 |
| | April | 11.5 | 1.0 | 69 | 14.9** | 1.4 | 39 | 17.9* | 2.0 | 14 | | | |
| 1991 | March | 12.3 | 1.1 | 62 | 16.3 | 1.5 | 62 | 20.4 | 2.1 | 61 | 23.1 | 2.6 | 33 |
| | April | 11.7 | 0.9 | 110 | 15.8 | 1.3 | 110 | 19.2 | 1.7 | 110 | 21.6 | 1.8 | 74 |

* Significantly smaller ($P<0.01$) than the same hatching month cohort in 1991.
** Significantly smaller $P<0.001$) than the same hatching month cohort in 1991.

(April-hatched cohort) were caught from late April to early May. This finding indicates that large numbers of small larvae were carried to the coastal fishing grounds after April 16 in 1990. The SST distribution in 8–9 May indicated that there was a substantial amount of intrusion of the offshore warm water after 23–25 April. Because the size of immigration to the fishing ground was smaller in this April-hatched cohort than in others, decline of growth in this cohort started earlier, at 13 d old (13.1 mm TL), compared with other cohorts from 20 to 21 d (19–20 mm TL) (Fig. 8).

Larval TLs at 20 d were 20.4 ±2.1 and 19.2 ±1.7 mm, as March- and April-hatched cohorts in 1991, respectively, which were comparable to the sizes at 20 d (19.1 ±0.6–19.4 ±1.6 mm) of the January- to March-hatched cohorts of *S. melanostictus* in the waters off Miyazaki in 1991 (Watanabe and Kuroki, 1997). In our current study, however, TLs of the 1990 larvae at 20 d ranged from 17.5 to 17.9 mm, significantly

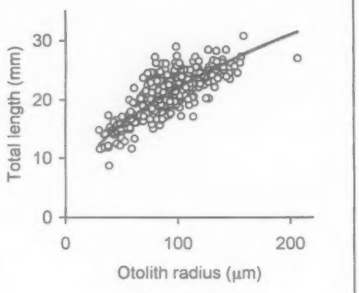


**Figure 7**

Allometric relationship of fish TL (mm) on OR (μm). $TL=2.34 \cdot OR^{0.482}$ ($r^2=0.685$, $n=312$, $P<0.001$).

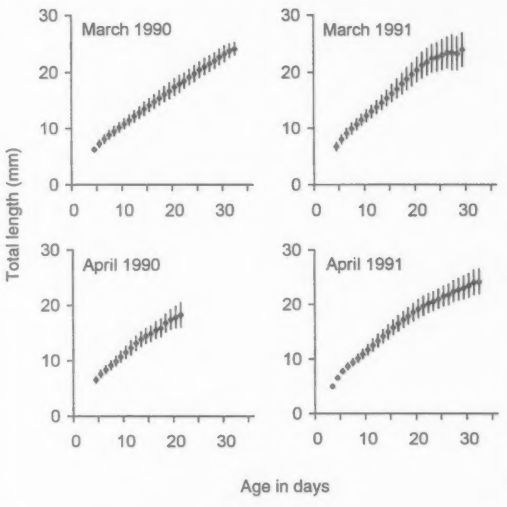


**Figure 8**

Backcalculated growth trajectories of sardine larvae hatched in March and April in 1990 and 1991. Small diamond represents mean TL at age and vertical bar one SD of the m

smaller than the sizes in 1991. The difference in growth rates between 1990 and 1991 could not be explained by SST because in our study area, slower growth was recorded in 1990, when SST was higher, than in 1991. Relative abundance of sardine *shirasu* in the coastal waters of central Japan in 1991 decreased to about 50% of the 1990 (Kishida et al., 1994). The catch of sardine larvae in 1991 declined to 41% of that in 1990 (Fig. 2). Funakoshi (1996) demonstrated that abundance of macrozooplankton was negatively correlated with the biomass of young-of-the-year sardines in Ise Bay (Fig. 1). He considered that large biomasses of young-of-the-year sardines resulted in a reduced abundance of macrozooplankton, followed by a decline in growth of the sardines through density-dependent processes. The greater population of sardine larvae in the *shirasu* fishing grounds in 1990 than in 1991 may have resulted in the slower growth of the sardine larvae in 1990. We need to study the density of food items for sardine larvae in the *shirasu* fishing grounds to examine if sardine larval growth is limited by food availability. We also need to know interspecific competition for food between sardine and anchovy larvae in a coastal ecosystem, because they coincided in April and May in our study area (Fig. 4).

The offshore Kuroshio frontal waters provide sardine larvae with sufficient foods. Nakata et al. (1995) calculated that the total food requirement of carnivorous macrozooplankters and sardine larvae was about 11% of the total production of small copepods (<1.0 mm prosome length) in Kuroshio frontal waters. This resulted in a higher feeding incidence in the early larval stage of *S. melanostictus* in the frontal waters compared with the inshore and offshore waters of the frontal area (Nakata, 1995). Juvenile *S. melanostictus* (32–48 mm fork length) collected in the Kuroshio frontal waters had stomach contents (mostly copepods and larvaceans) of 7–10% of wet body weight and were backcalculated to have grown at 0.8–0.9 mm/d in the larval stage (Watanabe and Saito, 1998). Perhaps the coastal waters in and around the *shirasu* fishing grounds are a less favorable feeding area for sardine larvae than the Kuroshio frontal waters.

## Acknowledgments

We thank Shigeo Funakoshi for comments on ecology of larval sardine and anchovy in the study area. Masao Bando sampled the sardine larvae in the fishing ports. Hisae Furukawa prepared and read otolith specimens. This work was supported in part by Grants-in-Aid from the Ministry of Agriculture, Forestry, and Fisheries (Biocosmos project, BCP-98-IV-A-8) and from the Ministry of Education, Science and Culture.

## Literature cited

**Anderson, J. T.**
**1988.** A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J. Northwest Atl. Fish. Sci. 8:55–66.

**Butler, J. L., K. A. Dahlin, and H. G. Moser.**
**1996.** Growth and duration of the planktonic phase and a stage based population matrix of Dover sole, *Microstomus pacificus*. Bull. Mar. Sci. 58:29–43.

**Campana, S. E.**
**1990.** How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. 47:2219–2227.
**1996.** Year-class strength and growth rate in young Atlantic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. 135:21–26.

**Campana, S. E., and C. M. Jones.**
**1992.** Analysis of otolith microstructure data. Can. Spec. Publ. Fish. Aquat. Sci. 117:73–100.

**Funakoshi, S.**
**1988.** Biological production mechanism of the Japanese anchovy and Japanese sardine shirasu in Suruga Bay and Enshu Nada. Bull. Japan. Soc. Fish. Oceanogr. 52:240–243.
**1996.** Variations in zooplankton abundance in Ise and Mikawa Bay. Kaiyo Monthly 28:142–149.

**Hayashi A, Y. Yamashita, K. Kawaguchi, and T. Ishii.**
**1989.** Rearing method and daily otolith ring of Japanese sardine larvae. Nippon Suisan Gakkaishi 55: 997–1000.

**Ishida, M., and H. Kikuchi.**
**1992.** Monthly egg productions of the Japanese sardine, anchovy, and mackerels off the southern coast of Japan by egg censuses: January 1989 through December 1990. National Research Institute of Fisheries Science, Yokohama, 86 p.

**Kasai, A.**
**1995.** Effect of variations in the Kuroshio and Oyashio currents on the egg and larval transport and recruitment of Japanese sardine (*Sardinops melanostictus*). Doctoral diss., University of Tokyo, Tokyo, 139 p.

**Kishida, T., Y. Katsumata, M. Nakamura, S. Yanagibashi, and S. Funakoshi.**
**1994.** An attempt for assessment on relative abundance of *shirasu*, larval Japanese sardine, off the Pacific coast of Japan. Bull. Natl. Res. Inst. Fish. Sci. 6:57–66.

**Kobayashi, M., T. Sugimoto, and T. Hirano.**
**1986.** Surface current patterns in the Kumano-nada and the Enshu-nada seas for different types of the Kuroshio paths based on GEK data - II for periods with large meander of the Kuroshio. Bull. Japan. Soc. Fish. Oceanogr. 50:2–11.

**Lo, N. C. H., P. E. Smith, and J. L. Butler.**
**1995.** Population growth of northern anchovy and Pacific sardine using stage-specific matrix model. Mar. Ecol. Prog. Ser. 127:15–26.

**Maritime Safety Agency.**
**1990.** Current profile. Quick bulletin of ocean conditions. No. 8–10. Maritime Safety Agency, Tokyo, 4 p.
**1991.** Current profile. Quick bulletin of ocean conditions, 8–10. Maritime Safety Agency, Tokyo, 4 p.

**Meekan, M. G., and L. Fortier.**
**1996.** Selection for fast growth during the larval life of Atlantic cod *Gadus morhua* on the Scotian Shelf. Mar. Ecol. Prog. Ser. 137:25–37.

**Mitani, I.**
**1987.** Echo sounder information on size range of anchovy larvae and juveniles forming a school in the shirasu fisheries ground in Sagami Bay. Bull. Japan. Soc. Fish. Oceanogr. 51:120–123.
**1990.** The biological studies on the larvae of Japanese anchovy, *Engraulis japonica* HOUTTUYN, in Sagami Bay. Spec. Rep. Kanagawa Prefect. Fish. Exp. Stn. 5:1–140.

**Muranaka, F.**
**1984.** Environmental variability and catch fluctuations of shirasu. *In* S. Funakoshi (ed.), Biological study on alteration of dominant larval species—Investigation of feeding conditions of early larvae, p. 195–220. Fisheries Agency, Tokyo.

**Nakata, K.**
**1995.** Feeding conditions of Japanese sardine in and near the Kuroshio examined from their gut contents. Bull. Natl. Res. Inst. Fish. Sci. 7:265–275.

**Nakata, K., H. Zenitani, and D. Inagake.**
**1995.** Differences in food availability for Japanese sardine larvae between the frontal region and the waters on the offshore side of Kuroshio. Fish. Oceanogr. 4:68–79.

**Smith, P. E., and S. L. Richardson.**
**1970.** Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175:1–100.

**Tsuji, S.**
**1983.** Study on recruitment mechanism of larval anchovy to shirasu fishing grounds in Sagami Bay based upon otolith daily increment analyses. Ph.D. diss., Univ. Tokyo, Tokyo, 157 p.

**Uotani, I., T. Iwakawa, and K. Kawaguchi.**
**1993.** Experimental study on the formation mechanisms of *shirasu* (postlarval Japanese anchovy) fishing grounds with special reference to turbidity. Nippon Suisan Gakkaishi 60:73–78.

**Watanabe, Y., and T. Kuroki.**
**1997.** Asymptotic growth trajectories of larval sardine in the coastal waters off western Japan. Mar. Biol. 127:369–378.

**Watanabe, Y., Y. Oozeki, and D. Kitagawa.**
**1997.** Larval parameters determining pre-schooling juvenile production of saury *Cololabis saira* in the northwestern Pacific. Can. J. Fish. Aquat. Sci. 54:1067–1076.

**Watanabe, Y., and Saito, H.**
**1998.** Growth and feeding of early juvenile Japanese sardines in the Pacific waters off central Japan. J. Fish Biol. 51:519–533.

**Watanabe, Y., H. Zenitani, and R. Kimura.**
**1995.** Population decline of the Japanese sardine *Sardinops melanostictus* owing to recruitment failures. Can. J. Fish. Aquat. Sci. 52:1609–1616.
**1996.** Offshore expansion of spawning of the Japanese sardine, *Sardinops melanostictus*, and its implication for egg and larval survival. Can. J. Fish. Aquat. Sci. 53:55–61.

**Zenitani, H., M. Ishida, H. Konishi, T. Goto, Y. Watanabe, and R. Kimura.**
**1995.** Distribution of eggs and larvae of pelagic fish species around Japan. Resources Management Research Report, ser. A-1:1–368.

# Transition from pelagic to benthic prey for age group 0–1 Atlantic cod, *Gadus morhua*

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Atlantic cod, *Gadus morhua* L., settle to the bottom early in life at standard lengths of 30 to 40 mm (Bowman, 1981; Hawkins et al., 1985; Hop et al., 1994; Methven and Bajdik, 1994). After settlement into benthic habitats, Atlantic cod continue to feed on pelagic prey but then shift to benthic prey. The rate at which cod make this transition has not been quantified at a level usable in trophic dynamic studies. To quantify the rate at which this shift in diet occurs, the volumetric proportion of pelagic prey in the diet of 98 juvenile Atlantic cod (age group 0 and 1) was measured. The data were used to evaluate a method that enables the calculation of food quantities with high accuracy for incorporation in trophic studies.

## Materials and methods

Juvenile Atlantic cod (ages 0 and 1) were collected at Bellevue, Trinity Bay, Newfoundland, from July to December 1989 and from August to October 1991. Refer to Methven and Bajdik (1994) for additional information on fish collection and the collection site. A sample of 98 group-0 (40.2–100.1 mm standard length [SL]) and group-1 (85.1–192.0 mm SL) cod were used. Stomach contents were analyzed by using prey volume to quantify the transition from pelagic to benthic prey.

For each individual, prey were identified to the lowest taxon possible, then counted. Displacement volume was measured for each taxon with a 5-mL graduated cylinder or a 200-µL micropipette. Prey volume was estimated as a proportion of the total volume of the pipette by using a millimeter ruler (200 µL measured 90.5 mm, i.e. 1 mm=approx. 2.2 µL). If a prey group was too small for volumetric displacement, measurements of length, width, and depth were taken for each individual prey item by using a dissecting microscope with an ocular micrometer. Measurements were made by using eye piece units and converted to millimeters to calculate volumes with geometric formulae (Table 1).

To evaluate the accuracy of calculated volumes, both displacement volume and calculated volume were measured for various prey items. Calculated volume tended to be higher than displacement volume by a constant rate, therefore linear regression was used to calibrate calculated volume against displacement volume. The intercept was not significantly different from zero ($P$=0.1453, $F_{[1,33]}$=116.43), therefore the parameters were re-estimated without the intercept ($P$=0.0001, $F_{[1,33]}$=258.02). The regression equation was

$$\textit{Displacement volume} = (0.72)(\textit{calculated volume}).$$

A correction factor of 0.72 was applied to all calculated volumes to obtain estimated displacement volumes.

The following references were used to identify invertebrate prey items and to determine whether they were benthic or pelagic with respect to habitat: Smith, 1964; Allen, 1967; Russell-Hunter, 1969; Schultz, 1969; Feeley and Wass, 1971; Meglitsch, 1972; Naylor, 1972; Bousfield, 1973; and Gardner and Szabo, 1982. Scott and Scott (1988) was used to identify vertebrate prey.

Percent pelagic prey in the diet was plotted against standard length to determine the relation between the two.

## Results

Both group-0 and group-1 cod fed on a broad range of prey. However, few occurred in large amounts

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Fish. Bull. 96:908–911 (1998).

**Table 1**

Prey shapes and volume formulae used in the study. spl = species length; spw = species width; spd = species depth.

| Prey type and species | Shape | Formula |
|---|---|---|
| Copepoda, Polychaeta, and shrimp and crab zoea | cylinder | $V = 3.14\ r^2 \times spl$<br>$r = spw/2$ |
| Amphipoda (straight) and Mysidicae | cylinder | $V = 3.14 \times r^2 \times spl$<br>$r = [(spw + spd)/2]/2$ or<br>$r = spw/2$ |
| Amphipoda (curved) | 1/2 cylinder | $V = 3.14 \times r^2 \times spw$<br>$r = spl/2$ |
| Crab (adult) | disc | $V = 3.14 \times r^2 \times spd$<br>$r = [(spl + spw)/2]/2$ |
| Crab (megalopa), Isopoda, and Ostracoda | box | $V = spl \times spw \times spd$ |
| Crustacean eggs and eyes | sphere | $V = 4/3 \times 3.14\ r^3$<br>$r = spl/2$ |
| Snail | circular cone | $V = 1/3 \times 3.14\ r^2 \times spd$<br>$r = [(spl + spw)/2]/2$ |

**Table 2**

Prey of age group 0–1 cod based on two measurement methods. B = Benthic, P = Pelagic, U = Unknown, Unid. = Unidentified. Mean number = number of individuals per stomach; mean volume = microliters per stomach.

| Method | Group 0 | | Group 1 | |
|---|---|---|---|---|
| Mean number | Calanoida (P) | 22.7 | *Jaera marina* (B) | 37.9 |
| | Harpacticoida (B) | 3.6 | Eggs (U) | 17.7 |
| | Unid. Copepoda (U) | 3.2 | *Gammarus oceanicus* (B) | 3.0 |
| | *Jaera marina* (B) | 2.7 | *Pontogeneia inermis* (P) | 3.0 |
| | *Pontogeneia inermis* (P) | 2.1 | Harpacticoida (B) | 1.2 |
| | Other (U) | 5.7 | Other (U) | 5.8 |
| Mean volume | Unid. Gammaridae (B) | 27.4 | *Crangon septemspinosa* (B) | 297.7 |
| | *Gammarus oceanicus* (B) | 24.9 | *Gammarus oceanicus* (B) | 98.4 |
| | Unid. Mysidacea (U) | 12.9 | *Jaera marina* (B) | 84.0 |
| | *Calliopius laeviusculus* (P) | 12.3 | *Urophycis tenuis* (B) | 62.5 |
| | *Pontogeneia inermis* (P) | 11.2 | Unid. Teleostei (U) | 42.6 |
| | Unid. Copepoda (U) | 10.7 | Unid. Decapoda (U) | 40.9 |
| | Calanoida (P) | 6.5 | Unid. Crustacea (U) | 31.1 |
| | *Jaera marina* (B) | 6.1 | Unid Polychaeta | 28.9 |
| | Other (U) | 100.0 | Other (U) | 317.1 |

(Table 2). Group-0 cod fed predominately on amphipod taxa (*Pontogeneia inermis*, *Gammarus oceanicus*, *Calliopius laeviusculus*, and unidentified Gammaridae) and copepod taxa (Calanoida and Harpacticoida). Predominate prey of group-1 cod were more diverse, including two amphipod taxa, an isopod (*Jaera marina*), invertebrate eggs, a shrimp (*Crangon septemspinosa*), white hake (*Urophycis tenuis*), and other unidentified teleosts, decapods, and polychaete worms.

Figure 1 indicates a rapid shift from pelagic to benthic prey at standard lengths of 60 to 100 mm. Four group-0 fish, represented by triangles in Figure 1, had <5% pelagic prey. Prey of these fish consisted of a large volume of unidentified prey and a single benthic prey item (e.g. one ostracod or one amphipod head). These fish were omitted in the calculation of the mean proportion of pelagic prey for the three size groups of cod in Table 3.

The rapid shift to benthic prey by juvenile Atlantic cod was related to body size. This change was quantified for use in food web computations. The diet of cod 40–59.9 mm SL was 98% pelagic prey, that of

cod 60–100 mm SL was 39% pelagic prey, and the diet of cod greater than 100 mm SL was 13% pelagic prey by volume. A simple explanation for this rapid shift is that the mouth opening of smaller size classes is too small to enable predation upon many benthic prey species. However, it is important for juvenile cod to settle to benthic habitats as soon as possible to find protection against predators. Therefore, they quickly shift to a benthic diet as soon as their gape size is large enough.

A series of hypotheses were examined to decide whether the rapid shift in diet was associated with conditions of the study: time of day, time of year, and breakdown of the thermocline. The pattern of change in Figure 1 was not due to time of day, because cod were captured primarily at night (18:10 to 04:30 hours). Data were then replotted by month of capture, which proved not to be responsible for the rapid shift in diet. Data were then replotted by date to investigate relation to the breakdown of the thermocline in mid-October. It was hypothesized that the thermocline keeps pelagic and benthic prey well separated (i.e. pelagic prey are unavailable to demersal juveniles). After the breakdown of the thermocline, the pelagic prey may occur lower in the water column. However, the change in percentage of pelagic prey did not change suddenly at the time of thermocline breakdown.

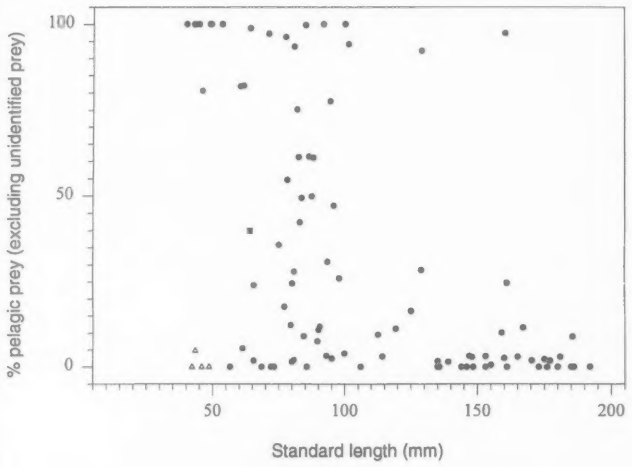


**Figure 1**

Percent pelagic prey (excluding unidentified prey) as a function of standard length (mm) for juvenile Atlantic cod. Four fish, indicated by the four triangles at the bottom left, had a single prey item identified.

**Table 3**

Percent pelagic prey in the diet of three size groups of juvenile Atlantic cod.

| Size group (mm) | Mean % pelagic prey | Standard error | Number of stomachs |
|---|---|---|---|
| 40–59 | 97.56 | 2.44 | 8 |
| 60–100 | 38.5 | 5.32 | 45 |
| >100 | 13.06 | 4.43 | 41 |

## Conclusions

The diets of group-0 and group-1 Atlantic cod at Trinity Bay, Newfoundland, are comparable to those of other areas (Daan, 1973 [northeastern Atlantic]; Arntz, 1974 [western Baltic]; Pálsson, 1980 [Iceland]; Bowman, 1981 [western Atlantic]; Keats et al., 1987 [eastern Newfoundland]; Paz et al., 1991 [Flemish Cap]; Keats and Steele, 1992 [eastern Newfoundland]; Hop et al., 1992, 1994 [northeastern Atlantic]). Demersal group-0 cod fed on both pelagic and benthic organisms, whereas group-1 cod fed more on benthic organisms as the principal components of the diet. The diets of group-0 and group-1 cod overlapped within a narrow range of body size (85.1 to 100.1 mm SL). Small pelagic prey, such as calanoid copepods, became less important in the diet of group-1 cod, whereas larger benthic prey items, such as decapods, amphipods, teleost fish, and polychaetes, became more important.

Results of the present study indicate that the ontogenetic shift from pelagic to benthic prey occurs within a narrow size range (from 60 to 100 mm SL) and hence more rapidly than previously believed (Daan, 1973; Bowman, 1981). This rapid shift may have been overlooked in previous studies that did not examine the diet of small cod in as much detail as in our study (i.e. volume in microliters of individual prey taxa). However, results of our study do not indicate that all cod less than 100 mm rely solely on benthic food. For example, it is well documented (e.g. Kohler and Fitzgerald, 1969) that, at particular times of the year, large juvenile and adult cod can forage far off the bottom on pelagic prey. One implication of this finding is that any investigation of cod recruitment variability, in relation to food supply, should include postsettlement stages.

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## Literature cited

**Allen, J. A.**
**1967.** Crustacea: Euphausiacea and Decapoda. Scott. Mar. Biol. Assoc. Ann Rep., Millport, 116 p.

**Arntz, W. E.**
**1974.** A contribution to the feeding of juvenile cod (*Gadus morhua*) in the western Baltic. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 166:13–19.

**Bousfield, E. L.**
**1973.** Shallow water Gammaridean amphipoda of New England. Cornell Univ. Press, New York, NY, 312 p.

**Bowman, R. E.**
**1981.** Food of 10 species of northwest Atlantic juvenile groundfish. Fish. Bull. 79:201–206.

**Daan, N.**
**1973.** A quantitative analysis of the food intake of North Sea cod, *Gadus morhua*. Neth. J. Sea Res. 6:479–517.

**Feeley, J. B., and M. L. Wass.**
**1971.** The distribution and ecology of the Gammaridea (Crustacea: Amphipoda) of the lower Chesapeake estuaries. Special Papers in Marine Science 2, Virginia Institute of Marine Science, Gloucester Point, VA, 58 p.

**Gardner, G. A., and I. Szabo.**
**1982.** British Columbia pelagic marine Copepoda: an identification manual and annotated bibliography. Gov. of Canada Fisheries and Oceans, Ottawa, 536 p.

**Hawkins, A.D., N. M. Soofiani, and G. W. Smith.**
**1985.** Growth and feeding of juvenile cod (*Gadus morhua* L.). J. Cons. Int. Explor. Mer 42:11–32.

**Hop, H., J. Gjøsœter, and D. S. Danielssen.**
**1992.** Seasonal feeding ecology of cod (*Gadus morhua* L.) on the Norwegian Skagerrak coast. ICES J. Mar. Sci. 49:453–461.
**1994.** Dietary composition of sympatric juvenile cod, *Gadus morhua* L., and juvenile whiting, *Merlangius merlangus* L., in a fjord of southern Norway. Aquacult. Fish. Manage. 25:49–64.

**Keats, D. W., and D. H. Steele.**
**1992.** Diurnal feeding of juvenile cod (*Gadus morhua*) which migrate into shallow waters at night in eastern Newfoundland. J. Northwest Atl. Fish. Sci. 13:7–14.

**Keats, D. W., D. H. Steele, and G. R. South.**
**1987.** The rôle of fleshy macroalgae in the ecology of juvenile cod (*Gadus morhua* L.) in inshore waters off eastern Newfoundland. Can. J. Zool. 65:49–53.

**Kohler, A. C., and D. N. Fitzgerald.**
**1969.** Comparisons of food of cod and haddock in the Gulf of St. Lawrence and on the Nova Scotia Banks. J. Fish. Res. Board Canada 26:1273–1287.

**Meglitsch, P. A.**
**1972.** Invertebrate zoology. Oxford Univ. Press, New York, NY, 834 p.

**Methven, D. A., and C. Bajdik.**
**1994.** Temporal variation in size and abundance of juvenile Atlantic cod (*Gadus morhua*) at an inshore site off eastern Newfoundland. Can. J. Fish. Aquat. Sci. 51: 78–90.

**Naylor, E.**
**1972.** British marine isopods. Academic Press Inc., London, 86 p.

**Pálsson, Ó.K.**
**1980.** On the biology of juvenile gadoids (age groups 0, I and II) in Icelandic waters. Meeresforschung Rep. Mar. Res. 28 (2–3):101–145. In German.

**Paz, J., M. Casas, and G. Perez-Gandaras.**
**1991.** The feeding of cod (*Gadus morhua*) on Flemish Cap. Northwest Atlantic Fisheries Organization (NAFO) SCR Document 91/31, serial 1911, 19 p.

**Russell-Hunter, W. D.**
**1969.** A biology of higher invertebrates. The Macmillan Company, New York, NY, 224 p.

**Schultz, G. A.**
**1969.** How to know the marine isopod crustaceans. W.C. Brown Company, IA, 359 p.

**Scott, W. B., and M. G. Scott.**
**1988.** Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219, 713 p.

**Smith, R. I.**
**1964.** Keys to the marine invertebrates of the Woods Hole region. Spaulding Company, MA, 208 p.

# Metazoan parasites as potential markers for selected Gulf of Alaska rockfishes

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Rockfishes (family Scorpaenidae) of the genus *Sebastes* constitute some of the most important groundfish resources in the northeastern Pacific Ocean. In Alaska waters, shortraker rockfish, *S. borealis*, and rougheye rockfish, *S. aleutianus*, are especially prized because their large size and red color make them valuable on the Asian market. Both species reside in offshore waters of the upper continental slope, and they often co-occur. Commercial catches of these two species in the Gulf of Alaska (GOA) have averaged 1700 metric tons per year during this period, and the estimated exvessel value of the fishery in 1991 (the most recent year with good economic data) was $3 million.

Knowledge of stock structure and the degree of mixing among populations is important for the rational management of commercially important marine species. At present, such information is lacking for shortraker and rougheye rockfish, and questions, such as whether these species perform lengthy migrations or whether discrete populations exist, are unanswered. Traditional tag-and-release experiments, which are often used to investigate stock structure, are not feasible for physoclistic fish such as rockfish because they cannot survive the barotrauma of initial capture. One alternative to human-implanted tags is the use of parasites as naturally occurring tags. Because necropsies of rockfishes are time consuming, we examined 100 fish of each species from the GOA to identify the feasibility of targeting selected parasites as tags for these species.

## Methods

Adult shortraker and rougheye rockfish were captured in the GOA in summer 1990 during an annual longline survey conducted on the upper continental slope by the National Marine Fisheries Service. Sampling covered five statistical management areas established by the International North Pacific Fisheries Commission (Zenger and Sigler, 1992): Shumagin, Chirikof, Kodiak, Yakutat, and Southeast (Fig. 1). The survey area extended from the Island of the Four Mountains (52°50'N, 170°W) eastward to Dixon Entrance (54°29'N, 134°W). Forty-five stations were sampled along the upper continental slope at depths from 150 to 1000 m. Of the 7836 rockfishes of various species caught in the cruise, a subsample of 20 rougheye and 20 shortraker rockfish (21 from Shumagin) from each management area were frozen on board for later necropsy. Total sample size was 101 shortraker and 100 rougheye rockfish from 21 stations.

In the laboratory, we conducted complete necropsies for metazoan parasites on an initial sample of 54 shortraker and 69 rougheye rockfish. As a result of preliminary analysis of the parasite data from these fish, we restricted the necropsies on the remaining 47 shortraker and 31 rougheye rockfish to examinations for the presence of gill and fin copepods, monogenetic gill trematodes, and visceral acanthocephalans. Identities of representative parasite specimens were verified by D. J. Whitaker of the Canadian Department of Fisheries and Oceans. Both prevalence (the proportion of fish with a given parasite) and intensity (the mean number of parasites per infected fish) were calculated for each management area.

Geographic trends were examined by grouping samples into the five management areas. Categorical analysis of variance (SAS procedure CATMOD; SAS Inst., 1989) was used to determine whether parasite prevalence differed among areas. For this analysis, the prevalence of a particular parasite was used as the dependent variable, and area and fish size as independent variables. For each fish species, median fork length was used to divide the areawide sample into two groups, hereafter referred to as large and small. Data on parasite intensity for each fish were divided by fish length to account for the possible influence of fish size on parasite intensity. Distribution of the intensity data was highly nonnormal, even after transformation. Therefore, intensity data were analyzed by using the nonparametric Mann-Whitney *U* test. These analyses allowed us to account for differences in fish size by area while test-

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Fish. Bull. 96:912–916 (1998).

ing for dependence of parasite prevalence and intensity by area. Fish size was chosen as a variable because the size of fish sampled in some areas differed, and fish size may influence parasite prevalence and intensity (Sekerak, 1975). A probability of 0.10 or less was judged to be statistically significant.

## Results

Seventeen species of parasites were found in our preliminary examinations: six species of copepod on the gills and fins and in the cephalic canal and nasal cavities; two species of monogenetic trematodes on the gills; three species of digenetic trematodes, two species of acanthocephalans, one species of cestode, and three species of nematodes in the viscera and mesenteries. All these species had been described previously from northeastern Pacific rockfishes, although parasites of shortraker and rougheye rockfishes in the western GOA had not been described previously. Many of the fish contained both larval nematodes *Contracaecum* spp. and *Hysterothylacium* spp. Because it is time consuming to separate these two genera, they were lumped together as a single species as *Contracaecum*-type spp. On the basis of these preliminary results, nematodes, digenes, and cestodes were eliminated from further examination. These helminths were infrequent in the samples or showed no difference between management areas. Further examinations focused on enumeration of copepods, acanthocephalans, and monogenes.

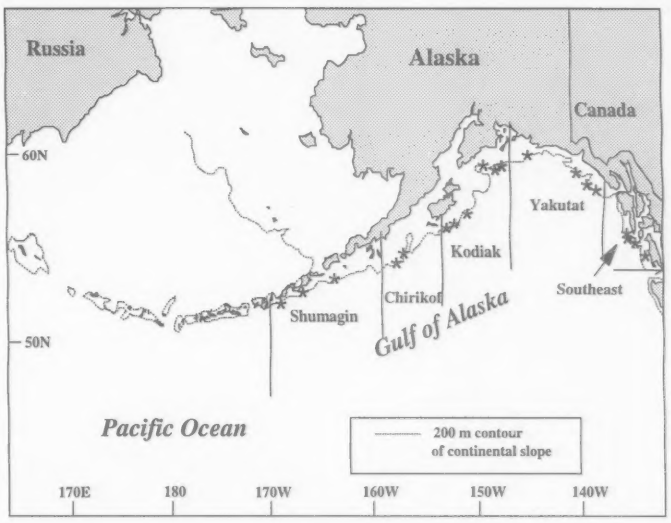


**Figure 1**

Northeastern Pacific Ocean, showing management areas of the Gulf of Alaska. Asterisks show sampling stations along the continental slope.

Prevalence (Table 1) and intensity (Table 2) of several parasite species differed distinctly among shortraker and rougheye rockfish populations in the GOA; they varied between areas, rather than being present in one area and absent in another. Three of these—*Neobrachiella robusta* (copepod), *Trochopus trituba* (monogenetic trematode), and *Corynosoma* sp. (acanthocephalan)—had the most distinctive changes in prevalence between areas. For shortraker rockfish, the highest prevalence of *N. robusta* and *T. trituba* parasites was in Kodiak samples. In rougheye rockfish, prevalence of all three parasites was significantly reduced in the Southeast. Several species of parasites were scarce in some areas and absent in others.

Of the six species of copepods in our survey, the gill copepod *N. robusta* was sufficiently prevalent to be potentially useful in stock discrimination studies. Prevalence of *N. robusta* differed significantly between areas for both shortraker ($P$=0.076) and rougheye ($P$=0.087) rockfish. *Neobrachiella robusta* was present in 80% of the Kodiak shortraker rockfish sampled as opposed to 35–55% of those sampled in the other four areas. Larger rougheye rockfish also had a significantly ($P$=0.028) greater prevalence of *N. robusta* than smaller specimens. Among shortraker rockfish, intensities ranged from a mean of 2.4 in Shumagin to 7.6 in Chirikof; the differences were also significant between areas ($P$=0.029), especially when corrected for the size of the fish ($P$=0.016). Among rougheye rockfish, however, intensity of *N. robusta* infection did not differ between areas ($P$=0.667). Mean infection levels were lower among rougheye rockfish than among shortraker rockfish for *N. robusta* but still averaged 1–5 parasites per fish.

Other copepods were prevalent in the GOA samples. *Naobranchia occidentalis* was found in shortraker rockfish from Chirikof, Kodiak, and Yakutat in low prevalence (10%) and in rougheye rockfish from Yakutat (20%), but not from other areas. Prevalence of *Chondracanthus pinguis* was above 40% in Yakutat and Southeast rougheye rockfish, but below 10% in all other samples. This difference, however, was a function of size rather than area. Prevalence was significantly greater ($P$=0.069) in large rougheye rockfish than in small ones. *Colobomatus kyphosus* was present in one shortraker rockfish from Yakutat and in two rougheye rockfish from Yakutat and Southeast. *Chrondracanthus triventri-*

**Table 1**

Comparison of parasite prevalence (proportion of fish having the parasite) by area for shortraker and rougheye rockfish in the Gulf of Alaska, 1990. The number of fish examined in each area is given in parentheses. Significance probability is given for a categorical analysis of variance model with area and fish size as main effects. Asterisks indicate $P < 0.10$.

| | Parasite prevalence | | | | | Significance probability | |
|---|---|---|---|---|---|---|---|
| | Shumagin | Chirikof | Kodiak | Yakutat | Southeast | Area | Size |
| Shortraker rockfish | (*n*=21) | (*n*=20) | (*n*=20) | (*n*=20) | (*n*=20) | | |
| *Neobrachiella robusta* | 0.48 | 0.55 | 0.80 | 0.35 | 0.40 | 0.076* | 0.724 |
| *Naobranchia occidentalis* | 0.00 | 0.10 | 0.10 | 0.10 | 0.00 | 0.980 | 0.493 |
| *Chondracanthus pinguis* | 0.10 | 0.05 | 0.10 | 0.10 | 0.10 | 0.971 | 0.301 |
| *Colobomatus kyphosus* | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.954 | 0.583 |
| *Trochopus trituba* | 0.62 | 0.60 | 0.86 | 0.55 | 0.45 | 0.081* | 0.262 |
| *Microcotyle sebastis* | 0.14 | 0.10 | 0.00 | 0.00 | 0.00 | 0.927 | 0.655 |
| *Corynosoma* sp. | 0.81 | 0.75 | 0.60 | 0.65 | 0.65 | 0.606 | 0.914 |
| *Echinorhynchus gadi* | 0.10 | 0.00 | 0.00 | 0.05 | 0.00 | 0.929 | 0.633 |
| *Clavella parva* | 0.62 | 0.80 | 0.90 | 0.30 | 0.20 | 0.013* | 0.370 |
| Rougheye rockfish | (*n*=20) | (*n*=20) | (*n*=20) | (*n*=20) | (*n*=20) | | |
| *Neobrachiella robusta* | 0.25 | 0.40 | 0.30 | 0.50 | 0.10 | 0.087* | 0.028* |
| *Naobranchia occidentalis* | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.897 | 0.823 |
| *Chondracanthus pinguis* | 0.10 | 0.00 | 0.00 | 0.40 | 0.50 | 0.152 | 0.069* |
| *Colobomatus kyphosus* | 0.00 | 0.00 | 0.00 | 0.10 | 0.10 | 0.960 | 0.165 |
| *Trochopus trituba* | 0.70 | 0.60 | 0.55 | 0.50 | 0.20 | 0.045* | 0.822 |
| *Microcotyle sebastis* | 0.15 | 0.00 | 0.00 | 0.15 | 0.00 | 0.906 | 0.895 |
| *Corynosoma* sp. | 0.80 | 0.75 | 0.75 | 0.75 | 0.20 | 0.001* | 0.283 |
| *Echinorhynchus gadi* | 0.00 | 0.00 | 0.15 | 0.00 | 0.05 | 0.674 | 0.356 |
| *Clavella parva* | 0.48 | 0.10 | 0.10 | 0.70 | 0.55 | 0.105 | 0.704 |

*cosus*, a common parasite in the nasal cavities of Canadian rockfishes, was largely absent from our samples, occurring in only a single specimen. The fin copepod *Clavella parva* was present in all areas, with increased prevalence among shortraker rockfish in the western GOA; this difference was significant ($P$=0.013) between areas. Although we examined all parasites in our sample, *C. parva* was never a candidate for separation, because fin parasites could be lost in capture and sampling.

The parasites showing the most potential for providing insights into the population structure for both species of rockfishes were the monogenetic trematodes *T. trituba* and *Microcotyle sebastis*, because of their high and low prevalences, respectively. Prevalence and intensity of *T. trituba* were significantly lower for rougheye rockfish in the Southeast (20% prevalence, mean intensity of 1.8) than in other management areas (50–70% prevalence, mean intensity of 2–9). For shortraker rockfish, prevalence and intensity of *T. trituba* were highest in Kodiak (86%, intensity of 16) and declined farther southward (45% and 8 in the Southeast, 62% and 4 in Shumagin). Both prevalence and intensities of *T. trituba* differed significantly among areas and did not depend on fish size. *Microcotyle sebastis*, common among many species of British Columbia rockfishes, was rare in the GOA.

The only internal parasite showing potential as a tag was *Corynosoma* sp., which, with the less common acanthocephalan *Echinorhynchus gadi*, infected nearly every Shumagin shortraker rockfish (90% infected with an acanthocephalan at an average of three *Corynosoma* sp. per fish). Most other shortraker rockfish were also infected (60–75%). Although prevalences of *Corynosoma* sp. in shortraker rockfish did not differ among areas, intensities and intensities/cm differed significantly ($P$=0.024 and 0.019, respectively) by area, largely owing to high numbers of *Corynosoma* sp. among Yakutat fish. Rougheye rockfish were also infected with *Corynosoma* sp. at 75–80%, except in the Southeast (20%; significantly lower [$P$=0.001] than in other areas).

## Discussion

Williams et al. (1992) proposed six criteria to assess the value of a parasite as a marker for stock separation. The parasite should differ in levels of infection across geographic regions, not be detached easily with handling, be easily assessed, have no harmful effect

**Table 2**

Comparison of mean parasite intensity by area for shortraker and rougheye rockfishes in the Gulf of Alaska, 1990. Range of intensity is in parentheses. Significance probability for differences in intensity and for intensity per cm fish length between areas is also given (Mann-Whitney *U* test). Asterisks indicate $P < 0.10$. Dash means insufficient number of infected fish to perform statistical tests. Int = intensity.

| | Mean parasite intensity (no. of parasites per infected fish) | | | | | Significance probability | |
|---|---|---|---|---|---|---|---|
| | Shumagin | Chirikof | Kodiak | Yakutat | Southeast | Intensity | Int/cm |
| Shortraker rockfish | | | | | | | |
| *Neobrachiella robusta* | 2.4 (1–9) | 7.6 (1–18) | 4.9 (1–12) | 4.4 (1–17) | 3.8 (1–8) | 0.029* | 0.016* |
| *Naobranchia occidentalis* | 0 | 1 (1) | 1 (1) | 4.5 (4–5) | 0 | 0.333 | 0.156 |
| *Chondracanthus pinguis* | 1 (1) | 3 (3) | 3.5 (3–4) | 1 (1) | 1.5 (1–2) | 0.131 | 0.189 |
| *Colobomatus kyphosus* | 0 | 0 | 0 | 1 (1) | 0 | — | — |
| *Trochopus trituba* | 3.8 (1–9) | 4.8 (1–9) | 16 (1–106) | 17 (2–89) | 8.3 (1–15) | 0.001* | 0.002* |
| *Microcotyle sebastis* | 1.3 (1–2) | 2.5 (2–3) | 0 | 0 | 0 | 0.133 | 0.083* |
| *Corynosoma* sp. | 3.3 (1–6) | 2.3 (1–12) | 2.7 (1–7) | 5.6 (1–26) | 3.7 (1–7) | 0.024* | 0.019* |
| *Echinorhynchus gadi* | 1 (1) | 0 | 0 | 3 (3) | 0 | — | 0.221 |
| *Clavella parva* | 1.2 (1–2) | 1.5 (1–5) | 1.5 (1–3) | 1.5 (1–2 | 1.3 (1–2) | 0.259 | 0.357 |
| Rougheye rockfish | | | | | | | |
| *Neobrachiella robusta* | 2.6 (1–4) | 2.7 (1–7) | 1.8 (1–4) | 5.2 (1–32) | 1 (1) | 0.667 | 0.478 |
| *Naobranchia occidentalis* | 0 | 0 | 0 | 1 (1) | 0 | — | — |
| *Chondracanthus pinguis* | 1 (1) | 0 | 0 | 9.6 (1–61) | 2.7 (1–5) | 0.235 | 0.211 |
| *Colobomatus kyphosus* | 0 | 0 | 0 | 3 (2–4) | 1.5 (1–2) | 0.333 | 0.121 |
| *Trochopus trituba* | 4.6 (1–19) | 1.7 (1–3) | 2.1 (1–8) | 9.1 (1–50) | 1.8 (1–3) | 0.023* | 0.069* |
| *Microcotyle sebastis* | 1 (1) | 0 | 0 | 1 (1) | 0 | — | — |
| *Corynosoma* sp. | 6 (1–17) | 5.1 (1–15) | 4.6 (1–8) | 5 (1–17) | 3.8 (1–7) | 0.973 | 0.916 |
| *Echinorhynchus gadi* | 0 | 0 | 1 (1) | 0 | 1 (1) | 1.000 | 0.180 |
| *Clavella parva* | 1.4 (1–3) | 1 (1) | 1 (1) | 1.8 (1–3) | 2.2 (1–5) | 0.427 | 0.443 |

on the host, have a long life span, and be present in only one part of the host. Based on coincident samples, it is clear that the monogenetic trematodes and copepods on the gills of the rockfishes in our study meet all these criteria, as do the visceral acanthocephalans. Gill parasites are protected by the operculum during capture, are sampled easily, and can be identified aboard ship. Notably, Leaman and Kabata (1987) previously proposed using *N. robusta* as a marker for separating stocks of *S. alutus* in British Columbia. Acanthocephalans, such as *Corynosoma* sp., also serve as excellent markers because they can be easily enumerated with a pepsin enzyme. In contrast, the lack of digenes and cestodes in the preliminary survey (probably due to regurgitation through barotrauma) makes these parasites poor candidates as tags for deepwater rockfishes. The nematodes are difficult to identify and their prevalences were similar throughout the Gulf of Alaska.

In addition to their potential use in determining stock structure, differences in prevalence between areas among the parasites in this study also give insight on differences in diet and parasite distributions. *Corynosoma* sp. is widely distributed in the northeastern Pacific Ocean as a parasite of marine mammals, birds, and fishes (Margolis, 1958); thus the different prevalences of Corynosoma sp. in our study were likely due to differences in diet rather than to parasite distribution. The intermediate hosts for *Corynosoma* sp. are amphipods; hence, the percentage of amphipods in the diet may be higher in the western part of the GOA than in the eastern part. Alternatively, rougheye and shortraker rockfishes may be more likely to consume infected amphipods than other prey items. In contrast, both copepods and monogenes have no intermediate host stage, and differences in parasitism between management areas probably reflect differences in parasite distribution or host habitat, rather than differences in diet.

Some of the parasite species reported in this study had very different prevalences than those reported for other species or locations of rockfishes. For example, *Corynosoma* sp. was less prevalent (<10%) in most species of British Columbia rockfishes (Sekerak, 1975; Stanley et al., 1992) than in our study. *Corynosoma* sp. may be more common in the GOA than in Canada or simply more prevalent in shortraker and rougheye rockfishes than in some other species. Sekerak (1975) examined 536 rockfishes of

26 species from British Columbia waters and the GOA: of the 40 *Corynosoma* sp. recovered, 12 were from rougheye rockfish and 23 were from the GOA.

Size did not appear significant in parasite prevalences except for *N. robusta* and *C. pinguis* infection in rougheye rockfish. Prevalences of several species of parasites, including *N. robusta*, differed significantly among fish of the same size. Nor was size a factor in parasite intensities, despite the observation of Sekerak (1975) that intensities of copepods and monogenetic trematodes increase with increasing fish size, largely due to increased gill surface area. Although the value of parasite tags would be enhanced by sampling similar-size fish, the statistically significant differences among areas, despite size variation, provide an indication of the potential power of parasite markers for these species.

In summary, the prevalence or intensity of *N. robusta*, *T. trituba*, and *Corynosoma* sp. may prove to be useful markers for population studies of GOA shortraker and rougheye rockfishes. It is interesting to note the major reduction in prevalence of all three parasites among Southeast rougheye rockfish. Possibly these fish may constitute a separate stock and support a localized fishery. More studies are needed to evaluate the effects of temporal and spatial variability on parasite prevalence and intensity, particularly among GOA rougheye rockfishes.

## Acknowledgments

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## Literature cited

**Leaman, B. M., and Z. Kabata.**
**1987**. *Neobrachiella robusta* (Wilson, 1912) (Copepoda: Lernaepodidae) as a tag for identification of stocks of its host, *Sebastes alutus* (Gilbert, 1890) (Pisces: Teleostei). Can. J. Zool. 65: 2579–2582.

**Margolis, L.**
**1958**. The occurrence of juvenile *Corynosoma* (Acanthocephala) in Pacific salmon (*Oncorhynchus* spp.). J. Fish. Res. Board Can. 15:983–990.

**SAS Institute, Inc.**
**1989**. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Inc., Cary, NC, 1,686 p.

**Sekerak, A. D.**
**1975**. Parasites as indicators of populations and species of rockfishes (*Sebastes*: Scorpaenidae) of the northeastern Pacific Ocean. Ph.D. diss., Univ. of Calgary, Alberta, 251 p.

**Stanley, R. D., D. L. Lee, and D. J. Whitaker.**
**1992**. Parasites of yellowtail rockfish, *Sebastes flavidus* (Ayres, 1862) (Pisces: Teleostei), from the Pacific coast of North America as potential biological tags for stock identification. Can. J. Zool. 70:1086–1096.

**Williams, H. H., K. MacKenzie, and A. M. McCarthy.**
**1992**. Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. Rev. Fish Biol. Fish. 2:144–176.

**Zenger, H. H., Jr., and M. F. Sigler.**
**1992**. Relative abundance of Gulf of Alaska sablefish and other groundfish based on National Marine Fisheries Service longline surveys, 1988–90. U.S. Dep. Commer., NOAA Tech. Memo. NMFS F/NWC-216, 103 p.

# Catchability and retention of larval European anchovy, *Engraulis encrasicolus*, with bongo nets

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Whenever data on larval abundance are used in producing estimates or indices of stock size or recruitment, accurate length distributions are required. Factors that might bias sample distributions must be taken into account and, in broadscale ichthyoplankton surveys, the effect of environmental and behavioral factors on the content of the samples or collections must be considered (Zweifel and Smith, 1981). The objective is to standardize sampling gear by applying correction factors to make samples comparable (Smith and Richardson, 1977).

A factor contributing to a possibly serious source of bias in larval fish collections is net avoidance. Larvae may be agile and capable of avoiding nets, with the general effect of inaccurate abundance and mortality estimates (Clutter and Anraku, 1968). Catchability varies mainly with light regime and larval length. The visual stimulus of the sampling device is believed to be of crucial importance. This is indicated by numerous investigations showing diel variation in avoidance, catches being significantly smaller during daylight than at night (Morse, 1989, and references therein).

A second factor affecting larval fish collections is extrusion of captured material through the net mesh. According to the "diagonal rule" (Saville, 1958; Smith et al., 1968), the maximum cross-sectional diameter of an organism must be greater than the mesh diagonal if it is to be fully retained. However, the "diagonal rule" is often too conservative (Lenarz, 1972; Colton et al., 1980).

We examined the effects of time of day (day-night-twilight), fish length, and ontogeny on the catchability of European anchovy (*Engraulis encrasicolus*), by using a 60-cm bongo net. We also investigated biases resulting from differential retention of larvae of different lengths, and, further, we examined the effect of correction of catch for net avoidance on estimation of mortality.

Information on catchability and retention of anchovy with plankton nets is very limited and results are often contrasting. Catchability of European anchovy with bongo nets is unknown. Regarding retention, there is just a single study indicating full retention of European anchovy larvae with the 0.333-mm net (Aldebert et al., 1975), whose data are contradictory to Lo (1983) who has estimated a 0.63 retention rate for northern anchovy, *Engraulis mordax*, larvae <4.0-mm with a 0.333-mm net.

## Materials and methods

From 1992 through 1995, five ichthyoplankton surveys were carried out in the Aegean Sea. A total of 474 stations were occupied in continental shelf and slope waters and areas characteristic of larval anchovy habitat (Fig. 1). Plankton and hydrographic sampling were performed at each station and cruise information is given in Table 1. Most stations were positive (i.e. at least one larva was captured).

A 60-cm bongo net sampler (Hydrobios) was used during all cruises. Mesh sizes on the sampler were 0.250-mm, and 0.500- or 0.335-mm, depending on the cruise (Table 1). The 0.250-mm mesh net is considered to retain clupeoid eggs and larvae completely (Aldebert et al., 1975; Colton et al., 1980; Leslie and Timmins, 1989).

Tows at two knots were double-oblique, within 5 m of the bottom to the surface, or from 120 m depth to the surface at deep stations. All tows consisted of a wire released to the desired depth and retrieved to the surface at standard speeds. The depth of the sampler could be monitored onboard at any time during the tow by means of a recording depthmeter attached to the sampler. Plastic codend buckets, with side windows of 155 $cm^2$ covered with net gauze, were used in an effort to minimize damage to larvae. Volumes filtered were calculated from a calibrated flowmeter in the mouth of each net.

All samples were sorted in the laboratory and larvae were identified to the lowest possible taxonomic level. Larval anchovies were counted and measured (notochord

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Fish. Bull. 96: 917–925 (1998).

or standard length to the nearest 0.1 mm). If more than 100 specimens of anchovy were captured in a tow, 100 randomly selected larvae were measured, and subsequent length frequencies were raised to the total number of larvae. Lengths were rounded to 1-mm length groups (e.g. 2–2.99: 2.5 mm).

Each station was assigned to day, night, or twilight hours according to the recorded time at the beginning of the tow. Twilight was designated as one hour before or after sunrise and sunset (Morse, 1989).

The analysis of retention and catchability was made after pooling all available data and, subsequently, estimating mean catch per length group. For example, mean catch per $m^2$ in each length category was calculated for 227 day, 146 night, and 84 twilight positive stations (Table 1). Pooling data over large temporal and spatial regimes integrates areal and temporal heterogeneity in distribution and abundance, and results of the analyses represent average conditions (Hewitt, 1981; Morse, 1989).

Negative stations (where no anchovy larvae were caught by either of two nets) were not included in our analysis (*sensu* Hewitt, 1981); we assumed that they represent samples drawn from outside larval habitat. Given that the number of negative stations was very low in our data set (17 out of 474, of which 10 were from one cruise, 92ANC2, Table 1), inclusion or exclusion of such negative stations was not expected to affect results.

Catches were standardized to numbers per $m^3$. This standardization is sufficient for comparing larval retention in paired different mesh-size nets but, in comparing daytime or twilight and nighttime catches, data had to be standardized to numbers per $m^2$, by using maximum tow depth and volume of water filtered (Houde, 1977). Mean standardized catches per length group were used to calculate the following ratios: day:night and twilight:night as well as 0.500-:0.250-mm mesh and 0.335-:0.250- mm mesh. Variances of ratios were approximated as in Somerton and Kobayashi (1989).

The calculation of mean catch and its variance followed the methods of Pennington (1983) for the delta-distribution of catch frequencies (see also Morse (1989)). The estimators based on the lognormal model (delta distribution) are more efficient for marine data than the usual sample estimators (Lo et al., 1992; Pennington, 1996). In particular, they provide reasonable estimates for data sets that contain isolated large catches.

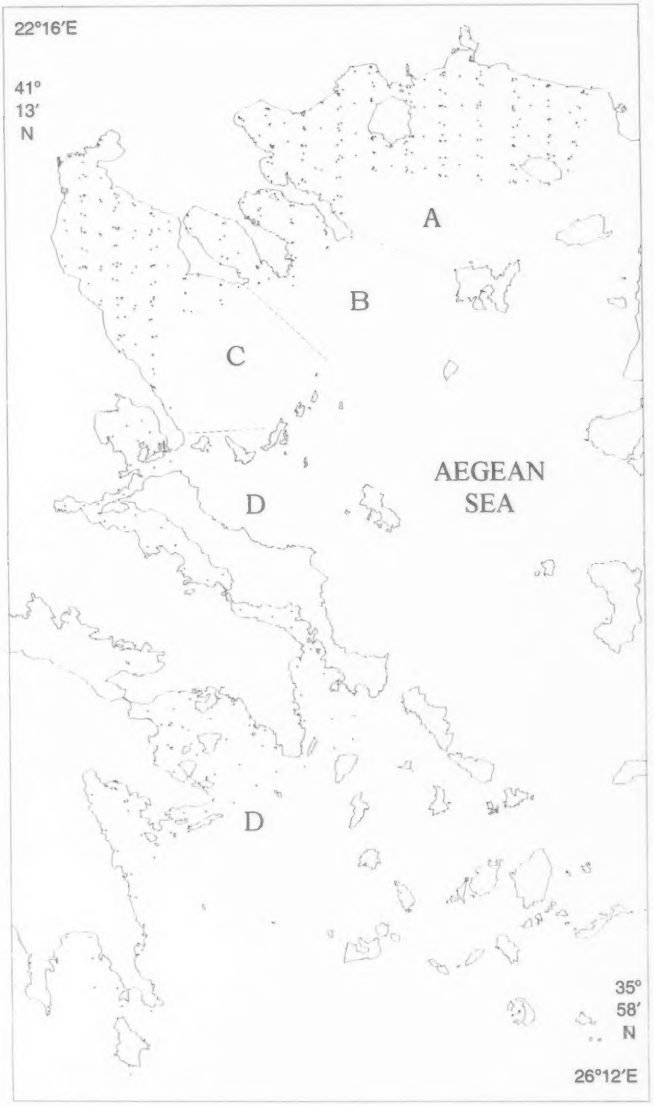


**Figure 1**

Map of the study area showing the location of sampling stations (black dots). A = Thracian Sea; B = Chalkidiki gulfs; C = Thermaikos Gulf; D = Gulfs of central and southeastern Greece.

Anchovies collected during 1995 were further sorted into yolksac, preflexion, flexion, and postflexion larvae. Eye pigmentation, functional mouth, and the formation of intestine (Regner, 1985; Palomera et al., 1988; Clarke, 1989) were used to distinguish yolksac from preflexion larvae. The flexion stage begins at initial notochord flexion and ends (postflexion stage starts)

**Table 1**

Cruise data. Subregions A, B, C (northern Aegean Sea), and D (central-southern Aegean Sea) are indicated in Figure 1. $n$ = number of stations. $n_p$ = number of positive stations. D = daytime stations. N = nighttime stations. T = twilight stations.

| Cruise | Date | Subregion | $n$ | $n_p$ | D | N | T | Mesh size (mm) | Larval abundance/m$^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 92ANC1 | 16 Jul–3 Aug 1992 | A-B-C | 117 | 117 | 54 | 44 | 19 | 0.250, 0.500 | 97.25 |
| 92ANC2 | 3–9 Sep 1992 | D | 80 | 70 | 30 | 26 | 14 | 0.250, 0.500 | 21.02 |
| 93ANC3 | 7–14 Jul 1993 | A-B-C | 110 | 110 | 62 | 30 | 18 | 0.250, 0.500 | 233.91 |
| 94ANC4 | 19–24 Jan 1994 | A-C | 46 | 43 | 16 | 18 | 9 | 0.250, 0.335 | 114.55 |
| 95EP1 | 15–30 Jun 1995 | A-B-C | 121 | 117 | 65 | 28 | 24 | 0.250, 0.335 | 69.17 |
| Total | | | 474 | 457 | 227 | 146 | 84 | | |

when the posterior margin of the upper hypural plate is at 90° from the notochord axis (Moser, 1996).

Additionally, to determine whether the inferences drawn regarding retention rates were plausible in terms of the "diagonal rule," we plotted maximum head width values against standard length based on measurements of 74 staged anchovy larvae (for a justification of using head width, see Colton et al., 1980). In postyolksac larvae, maximum head width was the maximum body width and was greater than any body-depth measurement (Somarakis, unpubl. data). This is not the case for yolksac larvae because of the bulk of the yolk sac. Yolk sacs, however, may be easily compressed or crushed, hence, we considered head width to be the significant dimension.

## Correction for net avoidance and mortality estimates

In studies on mortality of European anchovy the usual practice has been to correct catches for net avoidance by using day:night catch ratios and the methods available for northern anchovy (e.g. Palomera and Lleonart, 1989) because catchability of European anchovy has been unknown. As a final step of this study we estimated mortality during 1994 and 1995 cruises without correcting catches for net avoidance, as well as after correction using three different methods:

**Method 1** The correction for avoidance of the net during day-light was calculated by the sinusoidal function (Hewitt and Methot, 1982):

$$f1 = \frac{(1+DN_L)}{2} + \frac{(1-DN_L)}{2}\cos\left(\frac{2\pi t}{24}\right),$$

where $DN_L$ = the midday-to-midnight catch ratio of $L$-length larvae; and
$t$ = the hour of the tow.

$DN_L$ data used were those available for *E. mordax*.[1]

**Method 2** The same as method 1, $DN_L$ data used were those calculated for *E.encrasicolus* in the present study.

**Method 3** The sinusoidal function was not used. Length-specific ratios of day:night and twilight:night catches calculated in the present study were used to correct catches of day and twilight stations.

The correction for duration of each size class (Hewitt and Methot, 1982; Lo et al., 1989) was calculated from growth of postyolksac larvae in the sea measured by daily increments in otoliths (see also Somarakis et al., 1997a). Age-(micro-increment count)-at-length data and respective growth curves were available from 336 postyolksac larvae collected during the 1994 cruise, and 294 postyolksac larvae from the 1995 cruise. Significant spatial or interannual differences in growth of the European anchovy larvae in the Aegean Sea can be found, which cannot be explained by temperature differences (Somarakis et al., 1997a; Somarakis et al., 1997b). Thus, we estimated mortality for only the 1994 and 1995 cruises for which otolith data were available.

Mean corrected catches at length (i.e. length-specific daily production of larvae per m$^2$) were calculated based on the lognormal model (delta distribution—see above). Mortality was estimated for larvae >4-mm and <10-mm. The 3–3.99 size class was not used because it includes both yolksac and feeding larvae (the yolksac stage is characterized by different mortality rates than the feeding stage [Lo, 1985a; Lo, 1985b]). Mean length-specific daily production of larvae per m$^2$ with its age (calculated from the

[1] Lo, N. C. H. 1996. Southwest Fisheries Center, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038. Personal commun.

respective growth curve) constituted the database for nonlinear regression estimation of instantaneous mortality rate of postyolksac larvae in a simple exponential model,

$$P_t = P_0 \exp(-zt),$$

where $P_t$ = the daily production of larvae at age $t$;
$t$ = the age in days from the end of the yolksac stage;
$P_0$ = the daily larval production at $t = 0$; and
z = the daily instantaneous mortality rate.

The simple exponential mortality model fitted the data very well (Watanabe and Lo, 1988).

Mortality models resulting from the application of different methods of correcting (or not correcting) catches for light-induced net avoidance were compared by an analysis of the residual sum of squares (Chen et al., 1992).

## Results

### Estimates of mean catch

No larvae >12-mm were caught in any of the 227 day-time tows. Estimates of mean catch per length group (Table 2) indicated that catches of >10-mm larvae were very low and their coefficients of variation too great (generally >20%) to be useful in this study. Thus, our analysis was restricted to larvae <10-mm (see also Lenarz, 1972).

### Retention of anchovy larvae in the 0.335- and 0.500-mm mesh nets

Results of the catch analysis indicate that the 0.500:0.250-mm mesh catch ratio was less than one for larvae <7 mm but the 0.335:0.250-mm mesh ratio was not significantly different from one, at any length (Fig. 2).

Data of maximum head width against standard length of the 74 anchovy larvae, along with the mesh diagonals of 0.500-, 0.335-, and 0.250-mm, are presented in Figure 3. A comparison of head-width measurements with mesh diagonals indicates that the minimum lengths for complete retention in the 0.500- and 0.335-mm mesh nets were approximately 7.5 and 3.5 mm, respectively. The head-width measurements further suggest that to ensure full retention of larvae, it would be necessary to use netting with a mesh aperture of 0.250 mm or less. In terms of ontogenetic stages, the 0.335-mm mesh net is expected to retain all except the yolksac larvae, whereas the 0.500-mm mesh net seems to be inefficient for yolksac and preflexion larvae. It therefore appears that the "diagonal rule" is conservative for yolksac larvae of the European anchovy, when head width is used as the maximum cross-sectional diameter of the larva.

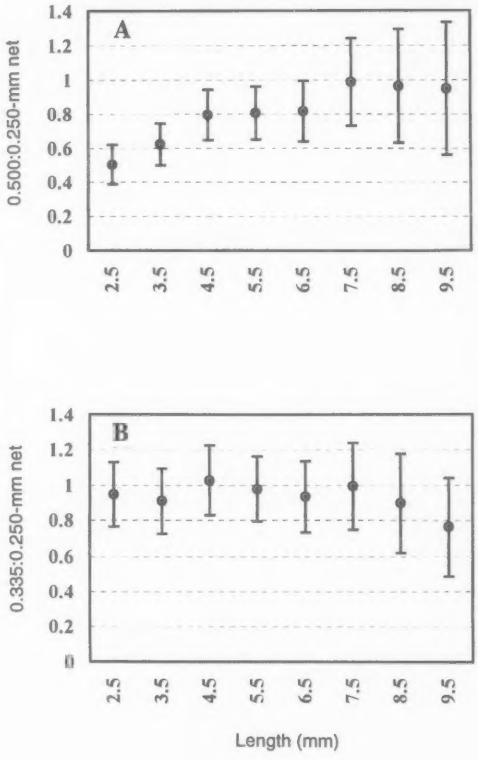


**Figure 2**

Catch ratios of 0.500:0.250 mm (**A**) and 0.335:0.250 mm (**B**) mesh nets as a function of larval length. Bars indicate 95% confidence intervals.

### Differences in day versus night and twilight versus night catches

Results of the catch analysis show a change in the day:night and twilight:night catch ratios at $L = 6.5$ mm (Fig. 4). In general, both ratios are practically one for larvae <6 mm, at which point they drop substantially, continuing to decrease more or less linearly thereafter. This decrease is stronger for the day:night than for the twilight:night catch ratio, the day:night ratio being significantly less than one from 6 mm onwards and greater than the corresponding twilight:night ratio. Thus, for larvae >6 mm, it seems

**Table 2**

Estimates of mean catch and their coefficients of variation (in parentheses), by length class.

| | Length (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 3.5 | 4.5 | 5.5 | 6.5 | 7.5 | 8.5 | 9.5 | 10.5 | 11.5 |
| 0.500:0.250 | | | | | | | | | | |
| 0.500 | 0.419 (0.079) | 0.378 (0.067) | 0.323 (0.062) | 0.264 (0.064) | 0.187 (0.072) | 0.136 (0.082) | 0.078 (0.115) | 0.054 (0.131) | 0.020 (0.200) | 0.012 (0.248) |
| 0.250 | 0.836 (0.084) | 0.607 (0.071) | 0.408 (0.069) | 0.329 (0.072) | 0.230 (0.080) | 0.139 (0.101) | 0.080 (0.128) | 0.056 (0.156) | 0.021 (0.233) | 0.012 (0.306) |
| 0.335:0.250 | | | | | | | | | | |
| 0.335 | 0.581 (0.067) | 0.453 (0.067) | 0.438 (0.068) | 0.325 (0.064) | 0.225 (0.077) | 0.182 (0.089) | 0.098 (0.109) | 0.062 (0.132) | 0.023 (0.201) | 0.013 (0.249) |
| 0.250 | 0.614 (0.068) | 0.498 (0.076) | 0.427 (0.068) | 0.332 (0.069) | 0.241 (0.074) | 0.184 (0.085) | 0.109 (0.110) | 0.082 (0.124) | 0.024 (0.232) | 0.015 (0.287) |
| day:night and twilight:night | | | | | | | | | | |
| day | 4.008 (0.067) | 2.900 (0.072) | 2.047 (0.075) | 1.582 (0.082) | 0.890 (0.093) | 0.587 (0.107) | 0.326 (0.132) | 0.201 (0.167) | 0.059 (0.297) | 0.043 (0.335) |
| night | 4.002 (0.085) | 2.775 (0.087) | 2.090 (0.088) | 1.512 (0.102) | 1.352 (0.101) | 1.003 (0.111) | 0.623 (0.129) | 0.496 (0.145) | 0.221 (0.191) | 0.099 (0.280) |
| twilight | 3.864 (0.115) | 3.098 (0.113) | 2.322 (0.114) | 1.683 (0.114) | 1.146 (0.129) | 0.748 (0.156) | 0.454 (0.186) | 0.319 (0.217) | 0.161 (0.319) | 0.101 (0.397) |

that catchability changes with varying light conditions and length. On the other hand, the "sudden" change in catchability at 6.5 mm coincides with the onset of the development of the tail, i.e. the flexion stage (Fig. 5).

### Correction for avoidance and its effect on mortality estimates

Estimates of length-specific larval production for the 1994 and 1995 cruises obtained by using the three different methods of adjusting data for net avoidance, or without any correction, were very similar (Table 3). Method 1, which was based on length-specific day:night catch ratio values calculated for *Engraulis mordax*, gave slightly greater values for larvae 4–6 mm, although differences were not statistically significant (overlapping confidence intervals).

Consequently, analyses of the residual sum of squares showed that the resulting mortality curves (Table 4) were not statistically different (1994: $F$= 0.361, $P$>0.5; 1995: $F$=0.780, $P$>0.5).

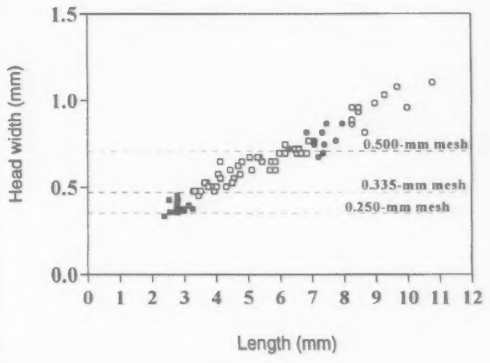


**Figure 3**

Relation between standard length and maximum head width for larval anchovy. The horizontal broken lines indicate the mesh diagonals of 0.500-, 0.335-, and 0.250-mm nets. Solid squares = yolksac larvae. Open squares = preflexion larvae. Solid circles = flexion larvae. Open circles = postflexion larvae.

## Discussion

### Retention of larvae

The results of this study indicate that the "diagonal rule" (maximum head width being considered as the maximum cross-sectional diameter of the larva) may adequately "predict" retention of anchovy larvae in the 0.500-mm mesh net but appears to be conservative in the case of the 0.335-mm mesh net. Specifically, yolksac larvae have a smaller head width than the diagonal of the 0.335-mm mesh net, but the latter seems to fully

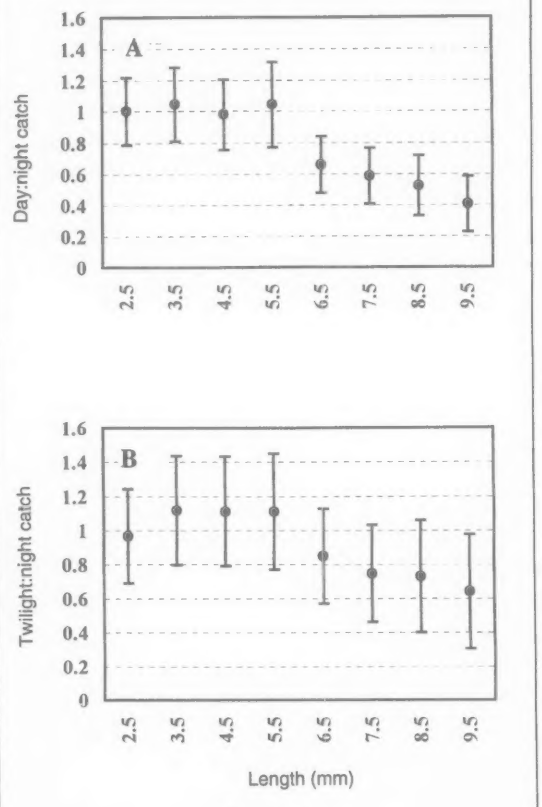


**Figure 4**

Day:night (**A**) and twilight:night (**B**) catch ratios as a function of larval length. Bars indicate 95% confidence intervals.

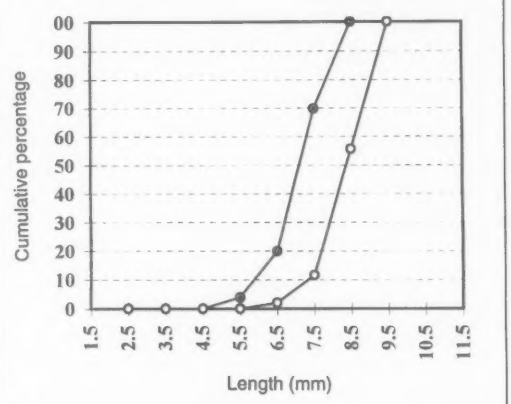


**Figure 5**

Cumulative percentages of flexion (solid circles) and postflexion larvae (open circles) as a function of larval length.

retain them. This is attributable to the fact that maximum head width is not the maximum cross-sectional diameter of the larva, because of the bulk of the yolk sac. Our method of towing (at low speed, with plastic codend buckets) did not seem to cause damage to yolk sacs and resulted in full retention of yolksac larvae by the 0.335-mm mesh. However, the degree of extrusion strongly depends on filtration velocity (Smith and Richardson, 1977; Colton et al., 1980). Thus, at high towing speeds yolk sacs may easily be crushed.

In his study on anchovy, Lenarz (1972) used depth of body at the insertion of the pectoral fin as the critical dimension to compare with mesh diagonal and concluded that the mesh "diagonal rule" was too conservative in the case of slowly towed nylon nets. However, body depth is not the critical dimension for clupeoids (Colton et al., 1980). The latter found no differences related to mesh size (0.253- and 0.333-mm mesh nets) in the length frequency distributions and abundances of larval herring in their collections at 1.5-knot towing speed. The mesh "diagonal rule" (with skull width as the critical dimension) was also conservative in their case, but for the length interval including only yolksac larvae. They found that most yolksac larvae caught in their slowly towed bongo nets, which were also equipped with plastic codend buckets, were undamaged.

We feel that at a low, constant towing speed (1.5–2.0 knots), bongo nets equipped with a 0.335-mm mesh net and plastic codend buckets are very efficient in retaining larvae of clupeoids.

## Net avoidance

Changes in catchability with varying light conditions and larval length are demonstrated in this study. These changes begin at notochord flexion. If visual detection of the net is the primary cue for net avoidance, flexion and postflexion European anchovy larvae show the expected relationship of night>twilight> day catches (Morse, 1989).

In another study, Murphy and Clutter (1972) compared catches of larval Hawaiian anchovy (*Stolephorus purpureus*) taken by conventional towed conical nets with catches taken by a miniature purse seine constructed of the same netting. The latter was considered to be a more effective sampler of the full size range of anchovy larvae than towed nets. In a way similar to the present study, larvae <6 mm long were captured with approximately equal efficiency by both the towed net and the seine (i.e. these larvae did not seem to avoid the towed net). For larger lar-

**Table 3**

Estimates of length-specific larval production for the 1994 and 1995 cruises (and their standard errors in parentheses) with different methods of adjusting data for net avoidance. Method 1 is based on day:night catch ratio values available for *Engraulis mordax*, and methods 2 and 3 are based on day:night and twilight:night values calculated in the present study (see text for details).

| | | Length (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| Cruise | Correction | 4.5 | 5.5 | 6.5 | 7.5 | 8.5 | 9.5 |
| 1994 | none | 1.984 (0.198) | 1.655 (0.198) | 1.169 (0.139) | 0.893 (0.127) | 0.518 (0.110) | 0.413 (0.093) |
| | method 1 | 2.142 (0.214) | 1.793 (0.212) | 1.281 (0.152) | 0.972 (0.138) | 0.569 (0.121) | 0.450 (0.102) |
| | method 2 | 1.984 (0.198) | 1.655 (0.198) | 1.231 (0.146) | 0.944 (0.134) | 0.553 (0.118) | 0.445 (0.101) |
| | method 3 | 1.984 (0.198) | 1.655 (0.198) | 1.258 (0.150) | 0.979 (0.140) | 0.573 (0.122) | 0.475 (0.109) |
| 1995 | none | 1.537 (0.111) | 1.235 (0.090) | 0.928 (0.080) | 0.690 (0.071) | 0.472 (0.052) | 0.341 (0.046) |
| | method 1 | 1.721 (0.029) | 1.409 (0.015) | 1.076 (0.058) | 0.722 (0.045) | 0.559 (0.046) | 0.399 (0.047) |
| | method 2 | 1.537 (0.111) | 1.235 (0.090) | 1.009 (0.086) | 0.681 (0.062) | 0.531 (0.057) | 0.390 (0.051) |
| | method 3 | 1.537 (0.111) | 1.235 (0.090) | 1.026 (0.087) | 0.706 (0.064) | 0.549 (0.059) | 0.412 (0.054) |

vae, the efficiency of the towed net decreased with larval length relative to the seine.

The change in catchability at flexion, observed in our study, can be attributed to a change in the swimming ability of larvae, which is associated with flexion. Batty (1984) found that Atlantic herring larvae, as they grow, change from an anguilliform mode of swimming to a subcarangiform mode of swimming. This change of swimming mode occurs as the caudal fin develops (at a body length of about 22 mm). Subsequently, Heath and Dunn (1990) using the large (5 $m^2$), high-speed LOCHNESS sampler, found that the day:night catch differential increased with body length for larval herring in the North Sea, with a maximum fivefold difference between day and night catches for larvae >25mm. In another study, Osse and van den Boogaart (1995) reported results similar to those of Batty (1984) for common carp (*Cyprinus carpio*). The morphological differentiation of the caudal fin, which begins at flexion and is accompanied by ossification of the caudal-fin rays and the caudal part of the notochord, closely parallels a change in the swimming mode from anguilliform to carangiform. Burst-speed capability, which is believed to determine, in part, the ability of a larva to avoid plankton nets (Hunter, 1976), might also change with the development of the tail.

**Table 4**

Estimated parameters of the exponential mortality models ($P_t=P_0\exp(-zt)$) for the 1994 and 1995 cruises. A different model was fitted for each different method of adjusting data for net avoidance (see text for details). $r^2$ = coefficient of determination.

| Cruise | Correction | $P_0$ | $z$ | $r^2$ |
|---|---|---|---|---|
| 1994 | none | 2.931 | 0.143 | 0.980 |
| | method 1 | 3.156 | 0.142 | 0.979 |
| | method 2 | 2.872 | 0.135 | 0.979 |
| | method 3 | 2.832 | 0.131 | 0.978 |
| 1995 | none | 2.195 | 0.136 | 0.992 |
| | method 1 | 2.463 | 0.135 | 0.989 |
| | method 2 | 2.139 | 0.126 | 0.989 |
| | method 3 | 2.109 | 0.121 | 0.989 |

Alternatively, changes in catchability may have a behavioral component. A substantial change in day:night catches of northern anchovy (*Engraulis mordax*) with bongo nets occurs at approximately 11 mm (Fig. 2 in Hewitt and Methot, 1982). Flexion in this species occurs at around 11 mm (Watson and Sandknop, 1996). Laboratory and field studies (Hunter and Sanchez, 1976; Hewitt, 1981; Hunter

and Coyne, 1982) have shown that, at about 11 mm, swimbladder inflation and schooling begins, whereupon patchiness increases rapidly. Our field observations of European anchovy indicate initiation of swimbladder inflation at flexion. It is therefore possible that schooling behavior also begins during this stage in *E. encrasicolus*.

School formation during the day and dispersion of schools during the night could result in low day:night catches for two main reasons: 1) larvae are less vulnerable to plankton nets when in a school than when reacting individually, owing to the reduction in the reaction distance of the organisms in the school to the approaching net; and 2) increased patchiness resulting from schooling during the day produces a larger number of negative tows.

In summary, field data on the European anchovy suggest the existence of an ontogenetic change in catchability with slowly towed plankton nets, which can be attributed to a respective change in the swimming ability, or a change in behavior (i.e. onset of schooling), or both.

To our knowledge, the present study is the first to examine retention and catchability in a larval fish not only in terms of length but also of ontogeny. Surprisingly, this study also shows that, when sampling is undertaken on a 24-h basis, bias that results from light-induced avoidance of the net may not be great enough to affect significantly estimates of daily larval production or larval mortality. However, when only daytime sampling is undertaken, mean catches of postflexion larvae may well be biased and correction factors may have to be applied.

Size selectivity due to net avoidance by larvae is expected to lead to an overestimation of mortality rate, because older larvae are underrepresented in relation to younger larvae. In a recent simulation study, Somerton and Kobayashi (1992) have shown that approaches usually taken to eliminate the selection bias from sampled length frequencies (i.e. division of sampled length frequencies by length-specific estimates of capture probability or elimination of the biased portion of the length distribution) may be only partially effective in reducing the bias in estimated mortality rates. The latter, as well as the present paper, suggests that the effectiveness of methods that attempt to correct for net avoidance is largely unknown. Their effectiveness has to be fully addressed.

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## Literature cited

**Aldebert, Y., A. J. Dicenta, Y. Marinaro, and C. Piccinetti.**
**1975.** Engins de pêches pour l' ichthyoplancton: essais comparatifs. Rev. Trav. Peches Marit. 39 (3):261–277.

**Batty, R. S.**
**1984.** Development of the swimming movements and musculature of larval herring (*Clupea harengus*). J. Exp. Biol. 110:217–229.

**Chen, Y., D. A. Jackson, and H. H. Harvey.**
**1992.** A comparison of von Bertalanffy and polynomial functions in modeling fish growth data. Can. J. Fish. Aquat. Sci. 49:1228–1235.

**Clarke, T. A.**
**1989.** Seasonal differences in spawning, egg size, and early development time of the Hawaiian anchovy or nehu, *Encrasicholina purpurea*. Fish Bull. 87:593–600.

**Clutter, R.I., and M. Anraku.**
**1968.** Avoidance of samplers. In D. J. Tranter (ed.), Zooplankton sampling. p. 57–76. UNESCO Monogr. Oceanogr. Methodol., vol. 2.

**Colton J. B., Jr., J. R. Green, R. R. Byron, and J .L. Frisella.**
**1980.** Bongo net retention rates as affected by towing speed and mesh size. Can. J. Fish. Aquat. Sci. 37:606–623.

**Heath, M., and J. Dunn.**
**1990.** Avoidance of a midwater frame trawl by herring larvae. J. Cons. Int. Explor. Mer 47:140–147.

**Hewitt, R.**
**1981.** The value of pattern in the distribution of young fish. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:229–236.

**Hewitt R. P., and R. D. Methot.**
**1982.** Distribution and mortality of northern anchovy larvae in 1978 and 1979. CalCOFI Rep. 23:226–245.

**Houde, E. D.**
**1977.** Abundance and potential yield of the round herring *Etrumeus teres* and aspects of its life history in the eastern Gulf of Mexico. Fish. Bull. 75:61–89.

**Hunter, J. R.**
**1976.** Behavior and survival of northern anchovy *Engraulis mordax* larvae. CalCOFI Rep. 19:138–146.

**Hunter, J. R., and K. M. Coyne.**
**1982.** The onset of schooling in northern anchovy larvae. CalCOFI Rep. 23:246–251.

**Hunter, J. R., and C. Sanchez.**
**1976.** Diel changes in swim bladder inflation of the larvae of the northern anchovy, *Engraulis mordax*. Fish. Bull. 74:847–855.

**Lenarz, W.H.**
**1972.** Mesh retention of *Sardinops caerulea* and *Engraulis mordax* by plankton nets. Fish. Bull. 70:839–848.

**Leslie, J. K., and C. A. Timmins.**
**1989.** Double nets for mesh aperture selection and sampling in ichthyoplankton studies. Fish. Res. 7:225–232.

**Lo, N. C. H.**
**1983.** Re-estimation of three parameters associated with anchovy egg and larval abundance: Temperature dependent incubation time, yolk-sac growth rate and egg and

larval retention in mesh nets. NOAA Tech. Memo. NMFS-SWFC-31, 32 p.

**1985a.** Egg production of the central stock of northern anchovy, *Engraulis mordax*, 1951-82. Fish. Bull. 83:137–150.

**1985b.** Modeling life-stage-specific instantaneous mortality rates, an application to northern anchovy, *Engraulis mordax*, eggs and larvae. Fish. Bull. 84:395–407.

**Lo, N. C. H., J. R. Hunter, and R. P. Hewitt.**

**1989.** Precision and bias of estimates of larval mortality. Fish. Bull. 87:399–416.

**Lo, N. C. H., L. D. Jacobson, and J. L. Squire.**

**1992.** Indices of relative abundance from fish spotter data based on delta-lognormal models. Can. J. Fish. Aquat. Sci. 49:2515–2526.

**Morse, W. W.**

**1989.** Catchability, growth, and mortality of larval fishes. Fish. Bull. 87:417–446.

**Moser, G. H. (editor).**

**1996.** The early stages of fishes in the California Current region. CalCOFI Atlas 33. 1505 p.

**Murphy, G. I., and R. I. Clutter.**

**1972.** Sampling anchovy larvae with a plankton purse seine. Fish. Bull. 70:789–798.

**Osse, J. W. M., and J. G. M. van den Boogaart.**

**1995.** Fish larvae, development, allometric growth, and the aquatic environment. ICES Mar. Sci. Symp. 201:21–34.

**Palomera, I., and J. Lleonart.**

**1989.** Field mortality estimates of anchovy larvae, *Engraulis encrasicolus*, in the western Mediterranean. J. Fish Biol. 35 (suppl. A):133–138.

**Palomera, I., B. Morales-Nin, and J. Lleonart.**

**1988.** Larval growth of anchovy, *Engraulis encrasicolus*, in the western Mediterranean Sea. Mar. Biol. 99:283–291.

**Pennington, M.**

**1983.** Efficient estimators of abundance, for fish and plankton surveys. Biometrics 39: 281–286.

**1996.** Estimating the mean and variance from highly skewed marine data. Fish. Bull. 94:498–505.

**Regner, S.**

**1985.** Ecology of planktonic stages of the anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the central Adriatic. Acta Adriat. 26:5–113.

**Saville, A.**

**1958.** Mesh selection of plankton nets. J. Cons. Int. Explor. Mer 23:192–201.

**Smith, P. E., R. C. Counts, and R. I. Clutter.**

**1968.** Changes in filtering efficiency of plankton nets due to clogging under tow. J. Cons. Int. Explor. Mer 32:232–248.

**Smith, P. E., and S. L. Richardson.**

**1977.** Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175, 100 p.

**Somarakis, S., I. Kostikas, N. Peristeraki, and N. Tsimenides.**

**1997a.** Fluctuating asymmetry in the otoliths of larval anchovy (*Engraulis encrasicolus*) and the use of developmental instability as an indicator of condition in larval fish. Mar. Ecol. Progr. Ser. 151:191–203.

**Somarakis, N. Peristeraki, and N. Tsimenides.**

**1997b.** Otolith microstructure and growth of larval anchovy (*Engraulis encrasicolus*) in the northern Evoikos Gulf (eastern Mediterranean). Proc. 5th Hellenic Symp. Ocean. Fish. Natl. Cent. Mar. Res., Athens, vol. 2, p. 51–54.

**Somerton, D. A., and D. R. Kobayashi.**

**1989.** A method for correcting catches of fish larvae for the size selection of plankton nets. Fish. Bull. 87:447–455.

**1992.** Inverse method for mortality and growth estimation: a new method for larval fishes. Fish. Bull. 90:368–375.

**Watanabe, Y., and N. C. H. Lo.**

**1988.** Larval production and mortality of Pacific saury, *Cololabis saira*, in the northwestern Pacific ocean. Fish. Bull. 78:601–613.

**Watson, W., and E. M. Sandknop.**

**1996.** Engraulidae: Anchovies. In H. G. Moser (editor), The early stages of fishes in the California Current region, p. 173–183. CalCOFI Atlas 33.

**Zweifel, J. R., and P. E. Smith.**

**1981.** Estimates of abundance and mortality of larval anchovies (1951-75): application of a new method. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:248–259.

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# Fishery Bulletin

## Guide for Contributors

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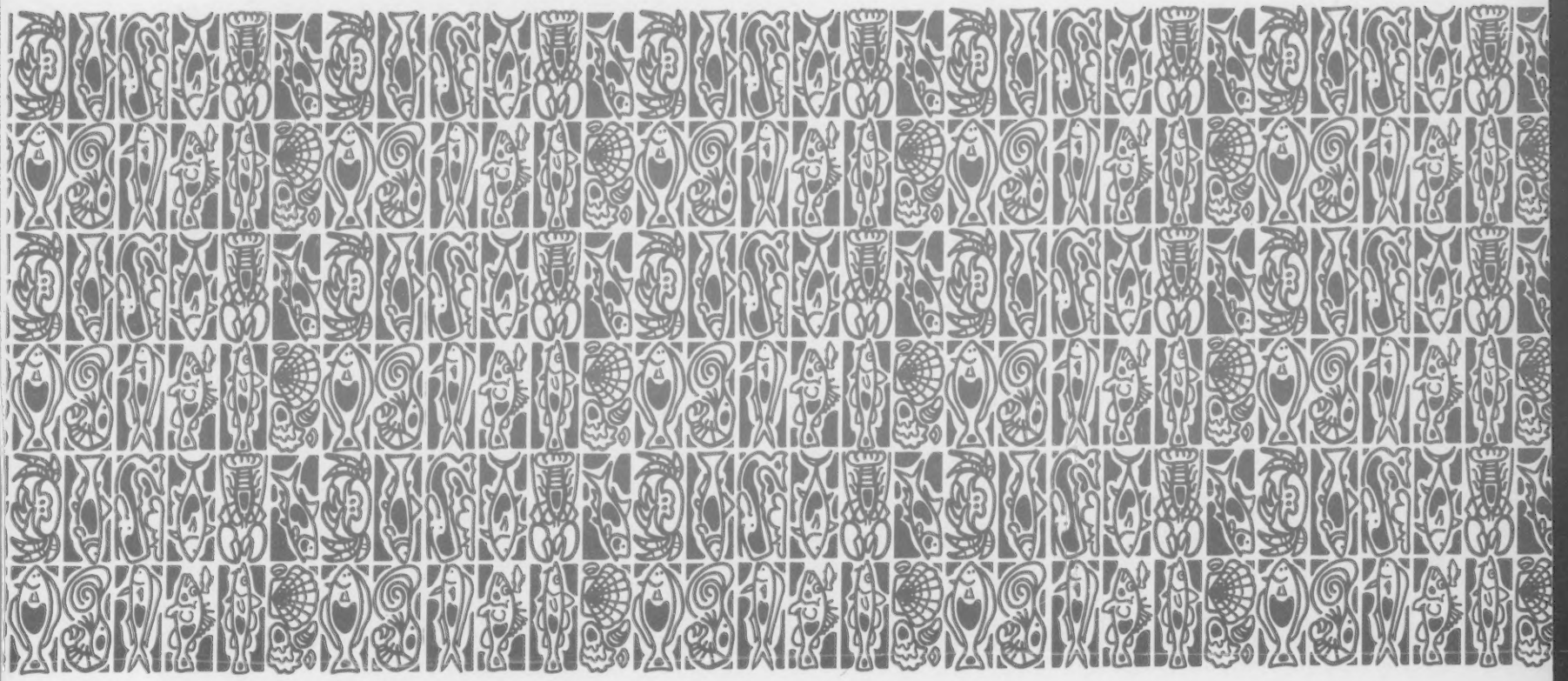

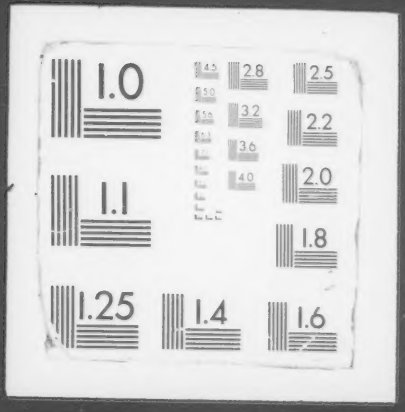
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